Genetic influence on brain volumes in psychosis.

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Genetic influence on brain volumes in psychosis

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This thesis is submitted for the degree of Doctor of Philosophy

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Abstract

This study explored the genetic influence of susceptibility genes on brain volumes in psychosis. More specifically, I explored whether certain allelic variations are associated with global brain volumes in subjects with psychosis, their relatives and in healthy controls. To increase the statistical power, I combined three different MRI scan datasets since a calibration study showed high intra-class correlations (ICC above 0.9) between MRI protocols (Chapter 3). I therefore included 535 participants (225 patients with psychosis, 130 relatives and 180 healthy volunteers).

In the first analysis I compared grey, white and whole brain volumes between patients and healthy controls (Chapter 5). Patients with schizophrenia spectrum psychoses showed smaller grey matter and larger white matter volume, and smaller whole brain volume than healthy controls. They also showed smaller grey matter volume than patients with bipolar disorder, who also had smaller white matter volume than healthy controls. In the second analysis, I estimated differences in brain volumes between relatives and patients and healthy controls (Chapter 6). Only patients with bipolar disorder showed smaller whole brain volume than their relatives. There were no significant differences in brain volumes between the relatives of patients with bipolar disorder or schizophrenia and the healthy volunteers. Finally, I evaluated the role of familiarity on these volumes (Chapter 6). In families with schizophrenia the family clusters accounted for 48% of the total variance in grey matter volume, for 27% of the total variance in white matter volume, and for 35% of the total variance in whole brain volume. In families with bipolar disorder the family clusters accounted for 48% of the total variance in grey matter volume, for 43% of the variance in white matter volume, and 22% of the total variance in whole brain volume.

In the second part of the thesis I explored genetic influence of 20 SNPs previously reported to influence brain morphometry in psychosis and in

healthy individuals, on these volumes (Chapter 7). I found evidence for an association between OLIG2_rs762178, MCPH_rs930557 and DTNBP1_rs1047631 and grey, white or whole brain volumes. However, this association did not survive multiple comparison corrections.

In conclusion, despite strong evidence of high heritability and familial influence on volumetric measures, I found not evidence of association between the selected candidate genes and these brain regions. These findings highlight the need to use large sample sizes when conducting this type of studies.

Acknowledgements

I am very pleased to be able to thank all of those who have helped me and supported me throughout the completion of this thesis.

First of all, I want to thank Sir Robin Murray who gave me the invaluable opportunity to become part of academic psychiatry in the UK. He has been a great inspiration in my career.

Special thanks are for my two very supportive and nourishing supervisors Dr Paola Dazzan and Dr John Powell. From them, I learnt research skills and analytical thinking during supervision time we spent together but more importantly, they have been an important source of encouragement by looking at their tirelessly dedication to research and education. I will always be grateful for all I have achieved during these years not just as a researcher but as a person.

This thesis would have not been completed without the support of Elvira Bramon, Colm McDonald, Conrad Iyegbe, Marta DiForti, Marco Piccioni and Timothea Tauloupoulou, Simone Reinders and Andy Simmons. Each of them has given me a piece of advice or a word of encouragement when I needed it. Their collaboration and genuine interest in supporting research fellows has showed me the importance of working in a professional and friendly environment.

My big, great, huge and more thanks to my mum, dad and sisters who always questioned “what is exactly that you are doing….?” and despite not much understanding of my obviously poor explanations, they always believe in me and encorage me in my career from the distance. They have been an increadible source of support and inspiration for my work.

Also to Esteban, who always believed and supported me. Finally, I want to say thank you to my friends Livia, Ana, Sonija, Maria, Natis and Barbi who accommodated themselves to see me and gave me strength and love in the little time I have been able to give them in the recent years.

To all of you THANK YOU!!!!

"Experience and imagination remain parallel tracks. The hypothesis flies; the fact walks"

Jose Ingenieros

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Chapter 1 Introduction

1.1 Introduction

Despite advances in neuroimaging and genetic research, the neurobiology of the two major functional psychotic illnesses; schizophrenia and bipolar affective disorder is little understood. This thesis describes my work analyzing magnetic resonance images (MRI) of the brain of patients, their unaffected relatives and healthy volunteers to determine the volumetric changes for grey and white matter associated with this illness and their relationship to an underlying genetic susceptibility.

1.2 Psychosis

Psychosis from the Greek ψυχή "psyche", for mind/soul, and -ωσις "-osis", for abnormal condition, means an abnormal condition of the mind. The first attempts to classify the illness were made by Kraepelin. He described dementia praecox where thought disorder, delusions and hallucinations were the prominent symptoms, in contrast with manic-depressive illness where mood changes were the prominent symptoms. Importantly, he also described that psychotic illnesses aggregate in families. Later on, this was further demonstrated in family studies of psychosis. In fact, family studies have estimated that the familiality of schizophrenia is between 41% and 87% (Gottesman and Shields 1982; Kendler et al, 1983; Cannon et al., 1998b; Cardno et al, 1999), while in bipolar disorder is between 73% and 87% (Cardno et al, 1999; Kendler et al, 1995, McGuffin et al, 2003). Nevertheless, the clinical distinction between these two major psychoses has always been less apparent. In the sections that follow, I will outline the classification of psychoses, the deviations in brain volume that have been described in these disorders, and the putative genetic approach to understanding psychotic illnesses.

1.3 Evolution of the classification of psychosis

From the first descriptions of psychotic symptoms, the definition of psychosis has been controversial. One of the first categorical classifications of the psychotic symptomatology was done by Kraepelin (Kraepelin; 1919). Kraepelin's dichotomous classification was based on evidence derived from the development of symptoms and clinical outcomes. He provided a description of "dementia praecox", in which thought disorder, delusions and hallucinations were the most prominent symptoms. He provided a second definition for manic-depressive illness, where mood changes were the most prominent symptoms. Later on, Bleuler reviewed Kraepelin's concepts and proposed the term schizophrenia, "split-mind" as it reflected better the clinical presentation and illness progress of patients suffering what it was called dementia precox (Bleuler, 1950).

Both Kraepelin and Bleuler subdivided schizophrenia into categories, based on differences in the most prominent symptoms and in prognoses. These classifications have been the basis for current categorical classification systems such as the Diagnostic and statistical manual of mental disorders 4th edition criteria, (DSM-IV, APA, 2000) and the International Statistical Classification of Diseases and Health Related Problems (ICD-10, WHO, 1992). In these systems these syndromes have been defined mainly into two major categories as schizophrenia and bipolar disorder with psychotic symptoms.

1.4 Current classification

In the ICD-10, the classification of psychosis as a syndrome falls into two major categories (and a similar classification is described in the DSM-IV), each including a number of other diagnoses:

1.4.1 Schizophrenia

F20 Schizophrenia: The main characteristics are distortions of thinking and perception, and affects that are inappropriate or blunted. These distortions are

accompanied by clear consciousness and intellectual capacity. The typical psychopathological phenomena include thought echo; thought insertion or withdrawal; thought broadcasting; delusional perception and delusions of control; influence or passivity; hallucinatory voices commenting or discussing the person's actions.

Schizophrenia presents with a sub classification depending on the presence of prominent symptoms and prognosis:

F20.0 Paranoid schizophrenia

F20.1 Hebephrenic schizophrenia

F20.2 Catatonic schizophrenia

F20.3 Undifferentiated schizophrenia:

F21 Schizotypal disorder Patients present with eccentric behaviour and anomalies of thinking and affect which resemble those seen in schizophrenia, though no definite and characteristic schizophrenic anomalies occur at any stage. An important characteristic is that there is no definite onset and course is usually those of a personality disorder.

F22 Persistent delusional disorders Includes a variety of disorders in which long-standing delusions constitute the only, or the most conspicuous, clinical characteristic.

F23 Acute and transient psychotic disorders This is a heterogeneous group of disorders characterized by the acute onset of psychotic symptoms with severe disruption of ordinary behaviour. Acute onset is defined as development of symptoms in two weeks or less. For these disorders there is no evidence of organic causation.

F24 Induced delusional disorder A delusional disorder where only one of the people suffers from a genuine psychotic disorder; the delusions are induced in the other(s) and usually disappear when the people are separated.

F25 Schizoaffective disorders In this category both affective and schizophrenic symptoms are prominent; therefore, do not justify a diagnosis of either schizophrenia or depressive or manic episodes.

F28 Other nonorganic psychotic disorders Delusional or hallucinatory disorders that do not justify a diagnosis of other disorder

F29 Schizphrenia Unspecified nonorganic psychosis

1.4.2 Mood Affective Disorders

F30 Manic episode The prominent symptoms are persistent elevation of mood, increased energy and activity, and usually marked feelings of well-being and both physical and mental efficiency. Additionally they could present with talkativeness, over-familiarity, increased sexual energy, and a decreased need for sleep. Irritability, conceit, and boorish behaviour may take the place of the more usual euphoric sociability. The disturbances of mood and behaviour might be accompanied by the presence of hallucinations or delusions.

F31 Bipolar affective disorder This category includes patients with two or more episodes of mood and activity levels with significant disturbance. These episodes have to include occasions of an elevation of mood, increased energy and activity (hypomania or mania) and on others of a lowering of mood and decreased energy and activity (depression). However, repeated episodes of hypomania or mania only are also classified as bipolar. Additionally, in order to be classified under the psychosis category they should present with psychotic symtoms i.e. hallucinations, delusions.

F32.3 Severe depressive episode with psychotic symptoms In this category, symptoms of depression should be present with hallucinations, delusions, psychomotor retardation, or stupor so severe that ordinary social activities are impossible.

Although over the years there have been attempts to classify psychotic illnesses based on their symptoms and prognosis, this has proven to be a difficult challenge. Although the two illnesses appear clinically distinguishable in the categorical classifications, the clinical boundaries between them often remain blurred because of a lack of pathognomonic symptoms. There is a clear overlap of symptoms in both illnesses, as demonstrated by studies using factor analysis of symptoms (Dikeos et al 2006, Demjaha et al., 2009) However, Dikeos and colleagues study (2006) reported mania as the best factor to distinguish between the two major psychotic disorders (Dikeos et al 2006). This finding supports the use of this approach when trying to distinguish schizophrenia from bipolar disorders.

With advances in research, several studies have been suggesting that both illnesses share some characteristics in terms of epidemiological and neurobiological risk factors (Murray et al., 2004; Craddock et al., 2005b). For instance, a small excess in winter birth has been shown to occur in both illnesses (Takei et al, 1992). Additionally, poor premorbid adjustment has been shown to be impaired during childhood in subjects who later on develop these illnesses but this is more noticeable in schizophrenia. This has been demonstrated by Cannon and colleagues in both retrospective (Cannon et al 1997) and prospective studies (Cannon et al 2002a). Interestingly, there is also a cross-over in risk, where for example a familial risk for schizophrenia increases the risk of developing an affective psychosis and vice versa (Kendler at al., 1993a,b; Valles et al., 2000; Cardno et al., 2002; Arajarvi et al., 2006).

Moreover, there are a number of genetic and clinical phenotypes such as sustained attention and working memory that overlap in patients with bipolar disorder and schizophrenia and their relatives, and these have been described in a recent meta-analysis and a review (Arts et al 2008, Ivleva et al., 2010). Furthermore, there is also evidence of an overlap in linkage and susceptibility genes between the two disorders (Bramon and Sham, 2001; Sklar et al., 2002; Craddock et al, 2005a; Lake, 2007).

In conclusion, findings from epidemiological and genetic studies show that there is an important overlap between the disorders described in classical dichotomous classifications of psychotic illnesses. However, more recently an effort has been made to find markers that could help distinguishing between these disorders. I will describe these in more detail in the following sections.

1.5 Brain development and its heritability

The focus of my thesis is the evaluation of brain volume differences in patients with psychosis, their healthy relatives and in healthy controls, and the evaluation of genetic contribution for these differences. During the past decade, new developments in schizophrenia research have led to understand psychosis as a brain disease. Moreover, changes in brain volumes have been reported to be present at the onset of the illness (Chua et al., 2007; Jayakumar et al., 2005). These findings suggest abnormalities during the brain development of patients with psychosis. Therefore, in order to understand how psychosis deviates from a trajectory of normality, it is important to understand first where differences between affected and unaffected individuals might lie. In the next section I will briefly describe the normal development of the brain in order to make inferences on its heritability, and on the genetic influence that might be implicated in the development of brain morphometric deviations in psychosis.

1.6 Normal brain development and heritability

Brain development and growth start in the pre-natal period and continue during childhood until early adulthood. There have been several studies describing the normal development of the brain and genetic influence on this process. By the age of 6 years, the human brain reaches 95% of the adult human brain volume (Giedd et al., 1999a) with grey and white matter volume increasing until early adolescence. After this period, grey matter volume (except temporal lobe area) starts to decrease while white matter volume continues to increase (Giedd et al., 1999a; Paus et al., 1999). Moreover, normal brain development follows different regional trajectories as shown by the work done by Giedd and colleagues (1996; 1999a). The authors described that the anatomic development of subcortical structures has been reported to be almost complete by late childhood while cortical regions having substantially longer development. In addition, some studies show that grey matter volume increases in late childhood and decreases during puberty following a consistent parietal-temporal-frontal lobe pattern (Giedd et al., 1999a,b). Furthermore, in longitudinal studies, white matter has been shown to increase until age 45 and to start decreasing after that age (Bartzokis et al., 2001). These findings are consistent with descriptions of brain volume changes in adulthood, when the whole brain volume decreases at the expenses of grey matter volume loss (Raz et al., 2004; Allen et al., 2005).

A number of studies have also described a dynamic variation in brain volume in adolescents in areas such as the frontal and temporal cortex, amygdala and hippocampus which seem to reduce their volume during this period (Sowell and Jernigan, 1998; Thompson et al., 2000). A well formulated theory explains this decline in volume as secondary to synaptic pruning (Huttenlocher 1979; Huttenlocher and Dabholkar, 1997). This theory describes brain volume changes as part of an adaptive process by which irrelevant connections are eliminated. Additionally, during adolescence there seems to be an increase in myelination in the limbic system pathways. During adolescence, when cognitive functions are enhanced, this change seems accompanied by

changes in brain activity in the frontal lobe, as seen with functional neuroimaging (Casey et al., 2000; Rubia et al., 2000).

Given that brain volumes are phenotypic expressions of genes, research in brain development in healthy people has looked at brain volume as a quantitative trait with high heritability (Thompson et al 2001a). In order to understand the expression of a certain phenotype, it is important to understand the concept of heritability as dependent on genes and environment. This can be expressed in the following formula (Fisher, 1918; Visscher et al., 2008):

Phenotype (*P*) = Genotype (*G*) + Environment (*E*).

The variance in the phenotype – Var (P) – is the sum of genetic effects as follows:

Var(*P*) = Var(*G*) + Var(*E*) + 2 Cov(*G*,*E*).

In a controlled experiment where environment can be fully controlled thus, Cov(*G*,*E*) can be held at 0. Therefore, heritability is defined as:

H^2 =Var (*G*)/Var(*P*)

This heritability would reflect all the genetic contributions to the phenotypic variance of a certain population. In order to estimate the heritability of certain phenotypes, familiality models take into account the variability of certain traits or phenotypes, such as brain volume, within families in comparison to the variability of the trait under study in not related individuals. In this model, both genetic and environmental factors are measured together. However, a more specific approach used in family and twins studies aims to assess the contribution of genetic and environmental factors separately. Here the heritability is described as the proportion of genetic variability over the total variance in a particular phenotype. Twins provide strong models to assess this. When using this approach, the heritability of brain volumes is estimated

by comparing the variation between monozygotic (MZ) pairs and dizygotics (DZ) pairs. It is argued that if the MZ pairs are more similar that the DZ pairs the trait measured (in this case, brain volumes) is due to genetic influence. The heritability of whole grey matter and white matter volumes has been estimated to be around 82% and 87% respectively (Posthuma et al. 2002). More recent studies have corroborated evidence of a high heritability in brain volumes, finding that the whole brain volume heritability is estimated around 70 %, while global grey and white matter volumes heritability is between 70 and 77% (Kremen at al., 2010).

Finally, it has been shown that the observed morphometric changes are likely to be due to changes in gene expression during human brain development as shown by Webster and colleagues (2002, 2006). The authors described changes in BDNF expression during neurodevelopment changes expression along the maturation of the prefrontal cortex, hyppocampus and temporal lobes. This finding further supports the theory of genetic influence in brain development and the need to investigate this further in psychosis.

1.7 Brain development in psychosis

There is increased evidence from MRI studies of brain structural abnormalities in psychosis (Wright et al 2000; Shenton et al 2001; Farrow et al 2005; Steen et al., 2006;, McDonald et al 2004; Kemptom et al 2008; Segall et al., 2009; Ellison-Wright and Bullmore 2010) and this will be discussed in the sections below. Moreover, there is strong evidence that these morphometric brain changes might be due to aberrant neurodevelopment as a result of interactions between genetic and environmental factors (Rapoport et al., 2005). This is also based on the view that schizophrenia is the behavioral expression of an abnormal neurodevelopmental course which begins before the clinical onset and is caused by a combination of environmental and genetic factors (Cardno et al., 1999a; Sigh et al., 2004). Keshavan and colleagues (1999a, 1999b) have proposed a model in which the brain development might be affected in subjects that later on develop

schizophrenia. This model suggests that environmental insults such as maternal viral infection in utero (Brown and Begg, 2005) could affect a crucial period of normal brain development and lead to the formation of abnormal neural networks (Keshavan, 1999a). Another crucial neurodevelopmental period, as described in the previous section, is during childhood and adolescence. In this period, the brain undergoes important developmental changes as shown in healthy adolescents (Giedd et al., 1999a) and it seems to be greatly controlled by genetic loading (Lenroot and Giedd, 2008). It has been proposed that genetic factor and/or environmental insults in this period might lead to loss of synapses (synaptic pruning) and brain plasticity (Keshavan et al., 1999b; Keshavan et al., 2005) underpinning the morphometric changes observed in psychosis. Studies on brain volumes in patients with childhood onset of schizophrenia (COS) have become of great value when trying to understand brain development in psychosis during this crucial time. Several studies in COS have shown significant grey matter volume loss and enlarged ventricles in comparison to healthy controls (Frazier et al., 1996; Rapoport 1997, 1999). Moreover, longitudinal studies have demonstrated that, in early onset schizophrenia (EOS), there are similar but more striking grey matter loss pattern than the ones found in healthy control (Gogtay 2004). One of the studies showed that in very EOS the initial deficit was significant parietal grey matter loss, which then progresses anteriorally into the temporal lobes towards the prefrontal cortices (Thompson et al., 2001b). These changes appear to be exaggerations of normal cortical development (Ropoport et al., 2005). In addition, the parallelism in brain changes over time between patients with schizophrenia and healthy controls in adult population was also shown in a recent study by Sun et al (2009). The authors showed that cortical brain reductions in patients and healthy controls have similar anatomical patterns albeit more significant in patient.

The neurodevelopmemtal theory for patients with bipolar disorder has been investigated but it has reached less conclusive results so far. There is some

evidence for possible abnormal brain maturation in bipolar patients from clinical pre-morbid neurobehavioral impairment. Cannon et al., (1997) reported a significant pre-morbid social impairment in bipolar patients in comparison to healthy controls. Moreover, a prospectively study from a British birth cohort study (van Os et al., 1997) found that patients that later on developed affective disorder presented with higher rate of speech defects; decreased psychomotor alertness and an excess of twitching and grimacing motor behaviors during childhood and adolescence. These findings suggest impairment in neurological development in bipolar disorder. From structural brain neuroimaging, increased amygdala volume in adult patients with bipolar disorder is one of the most consistently reported findings (Strakowski et al., 1999; Altshuler et al., 1998). However, reduction in amygdala volume has been found in adolescents (DelBello et al., 2004) and both adolescent and adult (Blumberg et al., 2003; 2004) patients with bipolar disorder. Therefore, emphasis has been put to evaluate the amygdala development in bipolar disorder. A study evaluated brain volume differences in adolescents with bipolar disorder (Chen at al., 2004). Although the differences were no statistically significant, the authors found a correlation between left amygdala volumes and age in bipolar patients which was not present in matched healthy controls. The authors suggest that this difference might reflect abnormal developmental pathway in bipolar disorder. However, there is still lack of evidence of progressive structural brain morphological changes from longitudinal studies which has very much supported the neurodevelopmental theory in schizophrenia. A recent comprehensive critical appraisal by Sanches et al., (2008) reviewed the evidence for the theory of neurodevelopmental disorder in bipolar disorder. The authors suggested that the evidence is weak and that the lack of positive findings might be related to use of medications as this has been shown to affect grey matter and whole brain volumes (Sassi et al., 2002). The authors concluded that abnormal neurodevelopment as a causal factor in bipolar disorder remains unclear and more research is needed.

In conclusion, the brain volume differences and the accelerated progressive reductions seen in patients with schizophrenia in comparison to healthy volunteers may be due to genetic factors leading to increased synaptic pruning, and supports the neurodevelopmental hypothesis of the illness. As for the bipolar disorders, the evidence for a neurodevelopmental theory still remians unconvincing.

1.8 Brain volume in psychosis

Starting in the last century, there has been much effort in trying to identify brain abnormalities as biological markers for psychosis. The initial approaches used in psychosis were either included post-mortem studies (Crichton-Browne, 1979; Southard 1910 and 1915; Jacobi and Winkler, 1928) or the application of invasive methods such as pneumonencephalography techniques (Haug et al., 1962; Jacobi and Winkler, 1928). Both these methods found enlarged lateral ventricles in individuals with schizophrenia. With the appearance of less invasive techniques such as X-rays and the development of Computer Tomography (CT), and later on Magnetic Resonance Imaging (MRI) the study of brain structure has progressed immensely. There is increased evidence from MRI studies of brain structural abnormalities in psychosis (Wright et al 2000; Shenton et al 2001; Farrow et al 2005; Steen et al., 2006; McDonald et al 2004; Kemptom et al 2008; Segall et al., 2009; Ellison-Wright and Bullmore 2010) and this will be discussed in the sections below. However, despite the technological progress, the identification of a reliable biomarker in psychosis has remained elusive. These could be partly due to the use of different MRI acquisition parameters across studies, including for example slide thickness (Filippi et al., 1998) or in methods used in measurements with variability in inter-rater and intra-rater reliability (Filippi et al., 1995). Nonetheless, recent automated computational methods based on Voxel Based Morphometry (VBM) have allowed a more reliable and faster estimation of structural brain differences (Ashburner and Friston, 2000)

among different patient populations such as schizophrenia (Wright et al., 1999; Meda et al., 2008), unipolar disorder (Du et al., 2012), autism (Abell et al., 1999), epilepsy (review by Keller and Roberts 2008) and healthy young people (Sowell et al., 1999). In the sections that follow, I will describe the most recent and consistent findings in structural neuroimaging MRI in schizophrenia and bipolar disorder.

1.8.1 *Schizophrenia*

Although there are limitations in the reliability of neuroimaging findings, due to heterogeneity of the samples evaluated and the variation in the methods used, a number of subtle volumetric changes have been consistently described in patients with schizophrenia when compared to healthy volunteers. One of the most consistent findings has been the observation of a reduction in whole brain volume and grey matter volume, as well as evidence of an enlargement of ventricular volumes in patients with schizophrenia (Wright et al 2000; Steen et al., 2006). A detailed literature review of studies that used region of interest approaches by Shenton et al. (2001) examined evidence provided for structural brain changes described in about a decade of research in schizophrenia. The most reliable findings identified with this approach have been enlarged lateral ventricles, reduced hyppocampal and amygdala volumes, and hemispheric asymmetries. Additionally, parahippocampal gyrus, superior temporal gyrus, prefrontal cortex, insula and cingulate gyrus have also been found to be reduced in volume in patients with schizophrenia when compared to healthy controls (Mc Carley et al., 1999; Hornea et al., 2005; Wright 1999 and 2000). These volumetric changes have been replicated in patients at their first episode of psychosis, when it is assumed that exposure to antipsychotic drugs would have been none or limited (Keshavan et al., 2005; Steen et al., 2006; Kuroki et al., 2006).

A meta-analysis also conducted in studies that used a region of interest approach concluded that there is a 2% reduction in whole brain volume (grey and white matter) in patients with schizophrenia when compared to healthy

controls (Wright et al., 2001). Several studies have found evidence of reduction in cortical grey matter volume in patients with schizophrenia when compared to controls (Wright et al 1999, Ananth et al 2002, Farrow et al 2005, Segall et al., 2009). Moreover, reductions in global gray matter volume have also been observed at the time of illness onset in medication naïve subjects (Salgado-Pineda et al., 2003; Jayakumar et al., 2005; Chua et al., 2007). These findings suggest that the loss of whole brain matter volume might be particularly due to grey matter loss (Vita et al. 2006). A more recent meta-analysis by Ellison-Wright and Bullmore (2010) concluded that there are increased basal ganglia and reduced thalamus volume. However, changes in the basal ganglia should be consider carefully as might be related to the medication effects as described by a systematic review carried out by Novaris and Dazzan (2009). The authors reported that antipsychotics seem to act more regionally in the basal ganglia rather than globally in the brain. Moreover, typical antipsychotics seem to be more responsible than with atypical antipsychotic in increasing the volume of the basal ganglia. They also reported that the data on differential effects of antipsychotic type on the cortex and thalamus still remains uncertain.

The evidence for structural neuroimaging and white matter volume has been less consistent. Reduction of whole white matter (Buchanan et al., 1998) and bilateral white matter reduction in frontal lobes (Buchanan et al., 1998; Paillere-Martinot et al., 2001) have been reported. The whole brain white matter volume decrease of about 1% of in schizophrenia patients in comparison to healthy controls have been reported by a meta-analysis (Wright et al., 2000). However these findings have not been consistently replicated for the whole white matter volume (Ananth et al 2002) or the frontal lobes (Baaré et al., 1999, Hirayasu et al., 2001 and Staal et al., 2000).

More recently evidence from VBM studies has been examined in the search of reliable marker for schizophrenia. A meta-analysis by Honea and collegaues in 2005 a described the reduction in left temporal lobe gyrus and left medial

temporal lobe as the most consistent finding in patients with schizophrenia when compared to healthy controls (Honea et al., 2005). Ellison-Wright and colleagues (2008) conducted a meta-analysis of VBM structural brain volumes in patients with schizphrenia. They reported reduction in grey matter in the basal ganglia-thalamo-cortical circuit (Ellison-Wright et al., 2008). In addition, these authors compared brain changes in first episode versus chronic schizophrenia patients. They found that cortical grey matter volume reductions seem more prominent in the chronic stages of the illness. Another meta-analysis by Chan et al. (2009) reviewed subjects with schizophrenia in their first episode and in their chronic stages. The authors described progressive brain volume changes in schizophrenia in the cortico-striato-thalamic circuit and showed that structural abnormalities in the fronto-temporal area are also evident in nonpsychotic individuals at high risk of developing schizophrenia. In addition, smaller gray matter volumes in the chronic group were more extensive. Taken together with the other evidence, these findings suggest that gray matter abnormalities become more extensive through first-episode and chronic illness. These progressive changes are important to consider when pooling together neuroimaging findings from first-episode and chronic patients with schizophrenia in multicentre studies.

Despite the report of some relatively consistent findings across studies of patients with schizophrenia, all studies comment on the heterogeneity of the methods used to evaluate structural brain volume differences. To advance evidence, it is therefore important to further investigate these findings in larger samples, with a consistent method that would limit the presence of methodological confounders.

1.8.2 *Bipolar disorder*

There is less consistent evidence of the brain alterations that are present in bipolar disorder. The studies carried out to date have often included heterogeneous samples of patients with affective symptoms, and either with or without psychotic symptom. In addition, many studies have included

patients with unipolar as well as bipolar disorder with psychotic symptoms. However, the studies that have narrowed their inclusion criteria for Bipolar Disorder I and II are still small in number when compared to the number of studies conducted in schizophrenia. One of the most consistent finding reported in bipolar disorder has been ventricular volume enlargement in patients when compared to healthy volunteers (McDonald et al 2004a, Kemptom et al 2008). Another frequently reported finding has been the presence of white matter abnormalities. Some of these appear to be qualitative rather than volumetric differences, and include an increase in periventricular and deep white matter hyperintensities (Altshuter et al 1995, Bearden et al 2001, Kempton et al 2008). Importantly, this has not been found at first onset of the illness (Strakowski et al 1993; Zanetti et al., 2008) but has been reported as in children and adolescents with the bipolar disorder compared to healthy volunteers (Pillai et al., 2002; Beyer et al 2009). In terms of structural white matter volume evaluations, smaller white matter volumes have been found in patients at their first episode of a bipolar illness in comparison to healthy volunteers (Vita et al 2009, Arnone et al 2010)

Deviations of regional brain volumes have been even more contradictory. Some studies have reported increased amygdala volumes as the most consistent finding (Altshuler et al., 1998; Strakowski et al 1999, Frodl et al 2002). There is no surprise that a brain structure involved in coding emotions would be affected in mood disorders. However, amygdala volume in adolescents with bipolar disorder has been reported as reduced in comparison to healthy volunteers (Chang et al 2005). Furthermore, these changes in the amygdale and subgenual prefrontal cortex have been supported by more recent studies in medication-free patients with major depression (Tang et al 2007) and at first-episode presentation of bipolar patients (Rosso et al 2007; Koo et al 2008).

This variability in findings has been attributed to factors such as sample characteristics, medication use and methodological heterogeneity. In recent years there have been a number of meta-analyses that have tried to aid researchers to narrow the brain deviations into the most consistent findings in affective psychosis. The clearest association has been reported for lateral ventricular enlargements (McDonald et al 2005, Kempton et al 2008, Arnone et al 2009), with less agreement for other brain structures. In the meta-analysis by McDonald et al. (2005), the authors found considerable heterogeneity in the volumetric deviations of the amygdala, thalamus and subgenual prefrontal cortex. Another area with inconsistent finding is the volume of the whole brain matter and the temporal lobe volumes and these deviations have been reported as possibly related to the duration of illness and the use of medication (Arnone et al., 2009). Also, the reduction in whole brain volume has not been found in first episode bipolar illness (Vita et al 2009), and this has been suggested to represent a marker of illness progression rather than of the illness itself.

In conclusion, it is not surprising that the critical appraisal carried out by Sanches et al. (2008) describes there is little evidence of consistent brain changes among patients with bipolar disorder.

1.8.3 *Schizophrenia and bipolar disorder*

There is still less evidence for brain volume differences between subjects with schizophrenia and those with bipolar disorder. Previous studies have found differences in volumes when comparing both groups; patients with schizophrenia showed statistically signifficant smaller grey matter volumes when compared to bipolar patients (McDonald et al., 2005; Yuksel et al., 2012).

Kempton and colleagues' (2008) meta-analysis of MRI studies in bipolar disorder attempted an additional analysis comparing brain findings in these

disorders. However, the authors found that there was a significant publication bias and therefore no conclusion could be drawn in this respect (Kempton et al 2008). In another meta-analysis, Arnone et al. (2009) showed that patients with schizophrenia present with smaller right amygdala volume and larger lateral ventricles bilaterally when compared to patients with bipolar disorder (Arnone et al., 2009). It is worth noting that studies using VBM were excluded from this analysis. Another meta-analysis by Ellison-Wright and Bullmore (2010) looked at the VBM method comparing both groups. The authors reported grey matter volume reductions in the anterior cingulate as characteristic of bipolar disorder, and volume reductions in the limbic system and neocortical structures as more specific to schizophrenia. However, there was substantial overlapping in the reduction of the insular volumes (Ellison-Wright and Bullmore, 2010). Moreover, patients with schizophrenia have been found to share the endophenotypes such as enlarged ventricles and P50 and the P300 auditory evoked potentials with subgroups of patients with bipolar disorder who also experience psychotic symptoms (Strasser et al., 2005; Potash et al., 2006). These findings support the concept that these disorders not only share common genes (Craddock et al. 2006, Potash et al., 2006; Craddock et al., 2009) and clinical presentation (Ivleva et al., 2008; Craddock and Owen 2010), but also the intermediate stage, brain deviations (Ivleva 2010).

1.8.4 *Influence of medications on brain volume*

Brain volume alterations in patients with schizophrenia have been described as present already from the first illness presentation (Keshavan et al., 2005; Kuroki et al., 2006; Steen et al., 2006; Vita et al., 2006). This has given rise to the notion that these alterations are not simply related to the influence of psychotropic medications such as antipsychotics, antidepressants or mood stabilizers, which have been reported to affect brain structure. The most common medications used in psychosis are antipsychotics. These mostly act on dopamine type 2 (D2) receptors, and are thought to achieve their

antipsychotic therapeutic effect by virtue of this blockade. A variety of studies has shown that antipsychotic drugs have direct effects on brain structure, and grey matter volumes in particular (Navari & Dazzan, 2009; Leung et al., 2011). One of the areas with highest density of D2 receptors are the basal ganglia which has been reported as increased in patients with schizophrenia exposed to neuroleptic medication, particularly first generation antipsychotics (Lang et al., 2004; Navari & Dazzan, 2009). When cortical grey matter volume is assessed, first generation antipsychotic medication seems also having an effect. Reduction of the pre-frontal cortex and medial temporal lobe has been shown to be affected more by first than second generation antipsychotic medication (Lieberman et al., 2005). In patients with bipolar disorder, Lithium is associated with increase grey matter volumes (Sassi et al., 2002; Bearden et al., 2007; Lyoo et al., 2010; Hallahan et al., 2011). A recent meta-analysis suggested associations between lithium treatment in patients with bipolar disorder and grey matter volume increases (Kempton et al*,* 2008).

1.9 Endophenotype approach to understand psychosis

One of the greatest challenges for psychiatry has always been to measure its illnesses in a reliable and valid manner. The aim has always been to categorize the symptoms to reach a diagnosis. One approach to try and identify biological markers that could be used to classify different illnesses has been the use of endophenotypes. The concept of endophenotype was introduced by Gottesman and Shields (Gottesman & Shields, 1973) as a theory to understand genetic concepts in schizophrenia. It is conceptualized as an internal phenotype evaluable by biological or microscopic tests.

A biological marker can be regarded as an endophenotype when it fulfills certain criteria as described by Gottesman and Gould (2003):

1) the endophenotype is associated with the illness in the population;
2) the endophenotype is heritable;

3) the endophenotype is primarily state-independent (independent of the illness activity);
4) the endophenotype co-segregate within families and
5) the endophenotype found in affected family members is found in non-affected family members at a higher rate than in the general population.

One of the difficulties in identifying endophenotypes in psychosis is the heterogeneity of the illness. There is evidence that schizophrenia and bipolar disorders share symptoms, susceptibility genes and biological markers (Craddock and Owen 2005a; Fischer and Carpenter 2009). Furthermore, there a number of endophenotypes that have been considered in psychosis such as neurocognitive deficits, whole brain volume, P50 and P300 event-related potential (Ivleva et al., 2010). Unfortunately, the study of these biomarkers has reported contradictory findings and no marker, so far, has been sensitive enough to be used to differentiate healthy controls from affected subjects. In the next section, I will describe the brain volume abnormalities that have been proposed as endophenotypes in order to help identify genes involved in a putative pathophysiology.

1.10 Brain volumes and unaffected relatives of patients with psychosis:

The inheritance of whole brain volume in schizophrenia has been described as between 66% and 97% (Peper et al., 2007; Rijsdijk et al., 2005). This has been further investigated in elderly twins by Pfefferbaum et al (2004) to additionally assess genetic influence on brain changes over time schizophrenia. The authors showed that the genetic influence in whole brain volume is still high, at around 80% in later life (Pfefferbaum et al., 2000). These results suggest that brain volume differences found in psychosis are under great genetic influence, making them appealing biomarker to be explored. However, in order to fulfil criteria as a biomarkers, the trait should be present in family members more than in the general population (Gottesman

and Gould, 2003). In this section I will highlight relevant findings on brain volumes in the unaffected relatives of patients with psychosis.

Some of the changes reported in schizophrenia such as, grey matter and ventricular volume differences, are shared by unaffected relatives of patients with schizophrenia, although to a lesser degree (Cannon et al 1998a; McDonald et al, 2002 and 2006). The comparison of offspring and siblings of patients with schizophrenia with healthy controls has shown reductions in the hippocampus volume (Kashavan et al 1997, Lawrie et al 1999; Johnstone et al., 2002). However, others have failed to replicate this finding (Schulze et al., 2003). A meta-analysis carried out by Boos and colleagues (2007) included different types of first-degree relatives; namely, siblings, monozygotic twins, dizygotic twins, parents, offspring and unspecified first-degree relatives. The authors' results support the initial findings on hippocampal volume reductions in healthy relatives of patients with schizophrenia in comparison to healthy controls. In addition, the authors reported significant whole grey matter reductions and enlarged third ventricles in the relatives group. Although ventricle volumes enlargement has been found in schizophrenia and their healthy relatives and proposed as an endophenotype (Mc Donald et al., 2002), more recently, the use of ventricular volume as an endophenotype in schizophrenia has been argued against, in view of its high vulnerability to environmental influences (Rijsdijk et al., 2005) and the high level of contradictory reports (Kremen et al., 2012). Regional differences between unaffected relatives of schizophrenia and healthy controls have been recently reported in brain volumes and grey matter thickness by Goghari and colleagues (2007). The authors reported volume and surface area reductions in the right cingulate gyrus; surface area decrease in the superior temporal lobe and, bilateral decrease in cingulate thickness. They also found relatives to have slight increase in gray matter volume in the left hemisphere, bilaterally in the parahippocampal gyri, and in the left middle temporal lobe (Goghari et al. 2007). Moreover, prefrontal cortex grey matter volume reductions has also

been found in relatives at high risk of developing schizophrenia in VBM studies (Job et al., 2003; Diwadkar et al, 2006). Additionally, although less prominently, areas such as medial temporal lobe have also been described to be reduced in healthy relatives of patients with schizophrenia (Lawrie et al., 1999; Keshavan et al., 1997; Job et a., 2003; Boos et al., 2007). However, a recent study by Boss and colleagues (2012) found no differences in grey or white matter volumes and cortical thickness between healthy siblings of patients with schizophrenia and healthy controls.

Twin studies have shown that co-twins with schizophrenia have about 2% smaller whole brain volumes than their non-schizophrenic co-twins; the latter, showed around 1% smaller brains than healthy control twins (Baare et al., 2001). In addition, a more recent twin study by van Haren and colleagues (2004) concluded that similarities for whole brain volume reductions were greater as pair members were more closely genetically related i.e. monozygotic twins > siblings > unrelated control subjects. Furthermore, the progressive brain change such as grey matter reductions, described in patients with schizophrenia has also been shown in their healthy co-twins and seem to be greatly influenced by a genetic component (Brans et al., 2008).

The evidence on structural brain changes in relatives of bipolar has been less extensively reported. Both bipolar patients and their first-degree relatives share a reduction in the anterior thalamic gray matter when compared to healthy colunteers (McIntosh et al., 2004). Other studies have shown reduction in pituitary volumes in bipolar patients and in their relatives when compared to healthy controls (Mondelli et al., 2008). However, in a study by McDonald et al (2006) relatives of patients with bipolar disorder showed no morphometric differences in comparison to healthy controls.

A study by McDonald et al., (2004) on structural brain endophenotypes and measures of genetic liability in families of patients with schizophrenia or

bipolar disorder, evaluated the genetic risks for brain volumes. The authors described a genetic risk for schizophrenia associated with bilateral fronto-striato-thalamic and left lateral temporal regions, while for bipolar disorder the risk was specifically associated with gray matter deficits in the right anterior cingulate gyrus and ventral striatum. White matter volume reduction in the left frontal and temporoparietal regions was present in both diseases and therefore, regarded as a generic endophenotypes.

All these findings suggest a genetic risk for psychosis is strongly related to brain structural alterations.

1.11 Brain volumes as endophenotypes in psychosis

Given the brain volume heritability in healthy people, its changes in the psychotic disease, and its reduction in unaffected relatives of patients with schizophrenia likely to be gene carriers, such as first-degree relatives or co-twins (Glahn et al., 2007), brain volume measures have become an appealing endophenotype in the study of psychosis. As reviewed earlier, structural MRI studies have proposed several brain structure variations that could be used as endophenotypes for psychosis. These have included for example, reductions in the whole brain and grey matter volumes, enlargement of cerebral ventricles and reduction of hippocampal volume (Prasad and Keshavan, 2008).

Despite strong evidence for brain volume changes in psychosis, there is still a poor understanding of the meaning and role of these findings in the development of psychosis. Although endophenotypes are a promising starting point, there are still difficulties in linking these abnormalities to the illness behavior. This difficulty lies in a number of factors, such as the heterogeneity of the clinical presentation of psychosis, the different methodologies used to assess different phenotypes, and the argument that psychosis is an illness with common genetic variants such as ZNF804A and CACNA1C (O'Donnovan

et al., 2009), each of weak effect as assessed in genome-wide association studies, in schizophrenia (O'Donovan et al., 2008) and bipolar disorder (Sklar et al., 2008; Ferreria et al., 2008). One additional major limitation lies in the power needed to detect subtle differences for each of these phenotypic or genetic variants.

Therefore, despite evidence from neuroimaging studies in psychosis, the question of finding biological markers that distinguish schizophrenia from bipolar disorder and healthy controls remains unanswered. Recently, more effort has been put in strategies that combine neuroimaging data acquired in different centers to address this pitfall and to increase the statistical power required for association studies. This approach will be described in the sections below.

1.12 Multicentre neuroimaging studies

Despite advances in MRI technology, there is still little understanding of the role of genes in brain volume. The main argument to explain this has been the small sample size and the lack of statistical power necessary to show a relationship, if one exists, between brain changes and genes. One obvious way to increase the statistical power of neuroimaging and genetic studies is to increase the sample size. While it is not difficult to pull data from different studies for genetic analysis, neuroimaging measures are more susceptible to variability in scanner properties and parameters settings during acquisition stages. In any neuroimaging study acquisition errors may occur by both technical issues such as spatial inhomogeneities (non-uniformity of the main magnetic field that can affect intensities and therefore tissue segmentation) or introduced by participants (different subjects position in the scanner). Some of these issues can affect the intensity of the single voxel, and hence affect the segmentation process for brain volume measurements. Some of these errors can be corrected for example by calibrating the scanner, or by following a particular protocol when positioning patients in the scanners. Even when

these precautions are taken, most sources of variability cannot be completely eliminated (Jovicich et al 2006). Nevertheless, many studies looked at the validity and reliability of using multi-centre neuroimaging data. There is evidence that the validity of combining neuroimaging data can be measured in a phantom (an artificial object of known dimensions and properties that is used to test or monitor an MRI systems homogeneity, imaging performance and orientation aspects). However, the introduction of humans into the scanner adds a "dynamic" variable (due to internal fluid movements and human movements) creating more image distortions than phantoms which needs to be assessed by test-retest and possibly corrected (Jovicich et al 2006). Therefore, the reliability of multi-centre neuroimaging data needs to be measured in repeated measurements in a phantom and in humans (Tofts et al 1998, VanHaren et al 2003, Jovicich et al 2006). Moreover, studies that use a small number of healthy volunteers repeatedly scanned on different scanners or after upgrades are also recommended to test reliability (Tofts, 1998; Han et al., 2006; Jovicich et al., 2006). These studies have assessed the reliability of multi-scanner data and given a solid base to understand the methods to reliably and accurately measure brain volumes when combining neuroimaging in muti-center studies.

In recent years, Voxel Based Morphometry (VBM) has become the leading method for analyzing large neuroimaging datasets. This method has the advantage of being almost fully automated, hence reducing the bias introduced by researcher variability (Ashburner and Friston 2000; 2005). On the other hand, to reliably apply this method, researchers still need to obtain good quality images that need to be successfully aligned in the initial step of this analysis method. In addition, the method is sensitive to differences in MRI scanners (Moorhead et al 2009).These can be corrected by using scanner specific priors (template images added in the neuroimaging pipeline to increase segmentation accuracy), but these do not solve the problems related to between-scanner differences in image characteristics and quality

(Ashburner and Friston 2000; 2005, Moorhead et al 2009). Therefore, when using VBM approaches such as Statistical Parametric Mapping (SPM) (Ashburner and Friston 2005), one of the most commonly used semi-automated methods for the analysis of brain structure based on VBM method, it is recommended that images should be obtained from the same scanner, so that variability in image quality is reduced.

There are only a handful of studies that have combined multiple neuroimaging datasets from patient samples. Most of them have looked at the reliability of combining neuroimaging data obtained from different scanners. However, they have all used different methods to process the neuroimaging and obtain brain volumes. One study looking at grey matter reductions in a cohort of patients with Alzheimer's disease using support vector machine (SVM) is an example of a supervised multivariate classification method (Vapnik, 1998; Bishop, 2006). The SVM method is highly dependant on spatial normalization pre-processing and therefore; the authors found that automated VBM methods can actually help overcome the errors introduced by the variability established when preprocessing a large neuroimaging sample from multiple scans (Kloppel et al 2008). However, it is important to mention that Alzheimer's disease tends to have more homogenous clinical populations than psychotic disorders.

In psychosis, a multi-centre study by Van Haren and colleagues (2003) evaluated brain volumes as predictors of outcome in a fist episode of psychosis. In order to assess the validity and reliability of the findings five healthy volunteers and a phantom were scanned at the three sites. They used a fully automatic methods based in histogram analysis (Schnack et al., 2001a and Schnack et al., 2004) followed by mathematical corrections depending on a calibration factor. The calibration factor is a constant for a fixed acquisition protocol, but which can differ for different protocols on different scanners. The authors found over and underestimations on grey and white matter separation

between scanners. However, by applying a linear scaling procedure on the histogram improved comparability between volume measurements of neuroimaging from the different scanners. In conclusion, this study also emphasizes the need of carrying out a calibration to ensure the test reliability on multicentre studies.

More recently a case control study conducted by Meda et al (2008) evaluated brain volume differences using VBM in SPM2. The brain scans were collected at four different sites. Therefore, to ensure reliability, authors created a study specific template. In this way, normalization of grey matter segments to a study-specific template ensures to better reflect grey matter distribution found within the study as it accounts for example any data inhomogeneities due to scanner differences. In addition, this approach ensures a minimal warping is required for coregistration as described in the optimized VBM method described by Good et al (2001). Moreover, scanner differences were tested by accessing a main effect of site to look at any grey matter differences that existed across scanners and using conjunction analysis to verify homogeneity of grey matter differences across sites. With this method, the authors replicated previous studies findings of decreased frontal, parietal, temporal grey matter volumes in schizophrenia. In addition, by increasing the sample size (400 participants) and therefore the statistical power to detect differences they also found reduced grey matter volume in areas such as the superior parietal lobule, caudate, rectal gyrus, and transverse temporal gyrus. However, they did not report whole grey, white or whole brain matter volume evaluation in this study. Their results suggest that VBM analyses of brain volume in psychosis can be improved by using larger samples.

In conclusion, despite the variety of neuroimaging processing methods, all the studies regarded the MRI multi-centre scan approach as a valid strategy to increase the power for neuroimaging studies in psychotic populations. To my

knowledge, there is no multicentre MRI study that has been conducted in bipolar disorder or in bipolar disorder and schizophrenia together using VBM.

1.13 Psychosis and genetics

Both schizophrenia and bipolar disorders have been reported to run in families by Kraepealin since the beginning of the 20th Century. Family, twin and adoption studies have addressed (described below) the familial segregation of schizophrenia and attempted to estimate the proportion of variance in vulnerability attributed to genetic and environmental factors. Heritability estimates for both disorders, schizophrenia and bipolar disorders, approach 80%. Therefore, large efforts have been put to identify the specific genes involved. During the last few years, advances in genetic technologies (e.g. dense genome-wide SNPs platforms and next generation sequencing), have enabled the parallel genotyping and sequencing of large number of individuals at a rate that was previously unconceivable. These advances have allowed the hypothesis-free search of genetic factors mediating vulnerability to psychosis by performing genome-wide association studies in large cases-controls samples including thousands of participants collected via international consortia. .

In this section I will describe the different genetic approaches that have been used to investigate the genetic influence in psychosis. Some studies have been based on the gene-environment interaction model, in which those psychotic illnesses are considered consequence of the combination of environmental and genetic factors. In order to explore the genetic influence in this interaction, genetic epidemiology has tried to answer main four questions as introduced by Faraone and Tsuang:

1- Is psychosis heritable?
2- What are the relative contributions of genes and environment?
3- What is the mode of transmission?

4- Which are the specific genes involved?

The first and second questions have been addressed by twin, family and adoption studies. Family studies have estimated the familiality of schizophrenia to be between 41% and 87% (Cardno et al, 1999; Kendler et al, 1983) while for bipolar disorder to be between 73% and 87% (Cardno et al, 1999; Kendler et al, 1995, McGuffin et al, 2003). Interestingly, there is also seem to be a cross-over, so that having a relative with schizophrenia increases the risk of developing bipolar disorder (Kendler et al, 1993a,b,c; Maier et al., 1993, 2002).

1.13.1 *Family studies*

These studies investigate the risk of developing the disorder of interest in relatives of patients as compared to the general population. However, the family study design is unable to disentangle genetic factors from environmental risk factors that are shared by members of the same family. Gottersman et al. (1991) showed that relatives of patients with schizophrenia have an increase risk of developing the illness. Moreover, the closer the relative the higher the risk is. In bipolar disorder, the risk of developing the disorder in the first degree relatives has been reported to be ten times higher in comparison to the general population. This evidence has found support in The Irish Roscommon Family Study. This study has shown that probands of several schizophrenia spectrum disorders and bipolar disorder segregate in families when compared with healthy controls and their relatives (Kendler et al, 1993a,b,c). Moreover, these findings have been recently replicated in a large Swedish study. In this study, it was reported that first degree relatives of patients with schizophrenia have a ninefold increased risk in comparison to the general population (Lichtenstein et al., 2009).

1.13.2 *Twin studies*

Twin studies have addressed the difficult question of how much is the contribution of either genetic or environmental factors in the development of psychotic disorders. Broadly speaking twin study models have been developed in order to explained genetic influence in the development of psychosis. In this way, if the disorder is driven by genetic factors the concordance rate in monozygotic twins should be higher than in dizygotic twins. These studies are designed on the rational that monozygotic twins should share 100% of their genes while dizygotic twins share approximately 50%. These give an estimated 2:1 ratio of genetic likeness to calculate the proportion of genetic risk of a given trait. Additionally, co-twins tend to share the same birth and nurture environment. The concordance rate of monozygotic twins is used to estimate the contribution of environmental factors, as they share the same genetic loading; while the difference between dizygotic twins is used to estimate the heritability as well as the environmental factors. In this way, if the familiar clustering is solely genetic, the concordance in the development of psychotic disorders should be near 100% in monozygotic twins. However, there are factors such as different genetic penetrance that affect the proportion of individuals who express a specific phenotype and environmental factors that also shape gene expression. All these additional factors that have an impact of gene expression make the assessment of genetic influence in psychosis very challenging. The concordance rate of monozygotic twins has been described in the range of 40 to 50% (Cardno and Gottesman, 2000; Sullivan et al., 2003).

Many studies have looked at the heritability of psychosis. In the Finnish national twin cohort study, described that the genetic factors that accounted for the disorder was 83% while the shared environmental factors were responsible for the remaining 17% (Cannon et al., 1998b) Similar results were found in the review of European twin studies by Cardno and Gottesman (Cardno and Gottesman, 2000). Interestingly, similar results were shown with the based on the Maudsley Twin Register with twins with schizophrenia

heritability of 83% and bipolar disorder heritability between 82% and 85% (Cardno et al., 1999). A recent meta-analysis by Sullivan and colleagues (2003) described the heritability of schizophrenia as 81%. Moreover, offspring of unaffected monozygotic co-twins carry the same risk of developing schizophrenia as the offspring of the affected co-twin (Gottesman and Bertelsen, 1989; Kringlen and Cramer, 1989). The heritability for bipolar disorder has been less often reported. However, it is estimated to be around 85% (McGuffin et al., 2003). These studies concur in suggesting that the genetic factors play a mayor role in the development of schizophrenia and affective psychosis.

1.13.3 *Adoption studies*

The aim of these studies is to assess the differences in risk between adoptive and biological relatives. There are mainly two methodological designs that are used to disentangle the effect of sharing the genetic predisposition and the nurturing environment.

a) The adoptees family method, which looks at the risk of developing the disorder in the adoptee individual. The most influential study has been the Danish Adoption Study (Kety et al., 1994). This study found that the risk of schizophrenia spectrum disorder was higher in the biological relatives of adoptees, who shared part of the genetic background but not the family environment, than in the relatives of control adoptees.

b) The adoptees method, in which the parent with schizophrenia is the proband. With this approach, The Finnish Adoptive Study of Schizophrenia re-examination of earlier work by Tienari et al., (1994) reported a 5.3% relative risk of schizophrenia in adoptees who had biological mothers with the illness (defined as high risk). In the low risk adoptees (defined as those whose biological mothers had either a non-schizophrenia-spectrum diagnosis or no lifetime psychiatric diagnosis) the relative risk was described at 1.7%. However, when comparing the risk with that of a broadly defined schizophrenia spectrum disorder in the mothers, the relative risk increased to

22.4% compared to those with low risk with 4.3% of morbid risk. In this study, there was not difference when comparing affective psychosis (Tienari et al., 2003).

In summary, adoption studies, using either of these approaches, provide evidence for the importance of genetic factors in the development of psychotic disorders.

1.13.4 *Mode of transmission*

Although its mode of transmission is still unknown, psychosis appears to be a complex multifactorial disorder related to a number of environmental factors and/or genes, which interact or co-act in causing the illness (Sullivan et al, 2003). This third question has been explored by segregation analysis. This is a preliminary method in genetic epidemiology to determine whether there is evidence that a major gene underlies the distribution of a given phenotypic trait. This analysis provides initial evidence whether a single gene has a major effect on a particular phenotypic trait only based on phenotypic information. Although these studies can show that a particular phenotype is related to the illness, they can not definitively prove that the trait is under the control of a single gene. The advantage of segregation analysis is to establish an association between a gene and trait which gives support to further investigate more in-depth studies such as linkage analysis.

It is now well establish that mode the of transmission of psychosis follows a multifactorial polygenic model (Gottesman and Shields, 1982) in which there is a large number of genes with small effect that in combination with environmental factors cause psychotic illnesses. The polygenetic component might explain the heterogeneity and overlap of symptoms we often encounter in clinical practice.

As discussed above, there is also increasing evidence that both schizophrenia and bipolar disorder co-segregate in families (Van Snellenberg 2009, Lichtenstein 2009) and share susceptibility genes (Craddock, O'Donnovan and Owen, 2005a, 2006; 2009; Moskvina et al., 2009; Schulze et al., 2012)..

Finally the fourth question is investigated by linkage and association genetic studies

1.13.5 *Molecular genetic analysis*

1.13.5.1 *Linkage studies*

Linkage studies are based on the theory that families affected with multiple affected individuals will show areas of the chromosomes that are affected. These studies serve as a base for exploring candidate genes in the region of the linkage signal and carry out association studies on alleles in the candidate genes There are a number of susceptibility loci that have been described as shared by schizophrenia and bipolar disorder including 13q32, 18p11 and 22q11-13 (Badner and Gershon, 2002; Levinson, 2003; Lewis et al; 2003). Chromosome 8 has been of great interest in the study of schizophrenia with a number of studies finding 8p23.1 and 8p21.3 to be in linkage disequilibrium (Blouin et al., 1998; Garver et al., 2001; Gurling et al., 2001; Suarez et al., 2006 Walss-Bass et al., 2006). More recently the advance for linkage studies is that it can be done in larger scale by genome wide linkage studies. A study carried out in 409 European and American ancestry families found showed evidence of possible linkage for schizophrenia in 8p23.3-p21.2 and 11p13.1-q14.1 in both populations and 4p16.1-p15.32 in the American ancestry population and 5p14.3-q11.2 European one (Suarez et al., 2006). As per bipolar disorders, chromosome 16p and 20 regions have been described to be implicated in the disease (Ross et al., 2008; Byelrley at al., 2011). In recent reviews, chromosomes 6q, 8q, 12q, 13q, 16p, 18p, 18q, and 22q have been also described in European families (Craddock et al., 2005a; Serretti and

Mandelli, 2008). Moreover, current data suggest strong association of the 3p21.1 locus and bipolar disorders (Vassos et al., 2012)

1.13.5.2 *Association analysis of candidate genes*

These studies evaluate the differences in allele frequencies between cases and controls at specific loci that are believed to be implicated in the aetio-pathogenesis of the disorders by previous knowledge. The hypothesis is that if the studied allele is involved in causing the disease, it should be overrepresented in cases. In this way, a specific gene and allele can be hypothesized to play a potential causal role in the disease. Additionally, these studies are design based in a specific phenotype or illness marker. Knowing the phenotype and the putative function of the candidate gene that plays a role in the selected phenotype, the association is calculated. Therefore, this study design tends to be in theory more powerful than linkage studies to detect differences as they based on *a priori* evidence of potential endophenotypes.

The selection of a specific endophenotype has proven difficult in psychosis due to the poor understanding of its ethiopathogenesis. However, association studies have focused to evaluate candidate genes either because they have implication in neurocircuits implicated in the disease (dopamine, serotonin, glutamate, and acetylcholine) or genes that are located in the previously identified areas of chromosomes by linkage studies.

In my study I included well established candidate genes as well as novel ones that could be implicated in brain volumes and psychosis. Some of the putative susceptibility genes identified for psychosis are thought to cause subtle structural variations in the brains of affected individuals, and might also influence brain structure in normal subjects (McIntosh et al, 2006). During the last few years, there has been remarkable progress in identifying risk genes for psychosis (reviewed in Kirov, O`Donovan and Owen, 2005, Craddock et

al., 2009). These include neuregulin 1 (NRG1) (Stefansson et al 2002), which has been replicated in Scotland (Stefansson et al., 2003), China (Tang et al., 2004) and other populations; dysbindin (DTNBP1) (Straub et al, 2002 and Kirov et al 2004); and disrupted in schizophrenia (DISC1) (Miller, KJ, et al, 2000) as susceptibility genes. Other genes such as catecholamine-O-methyl transferase (COMT) (Egan et al., 2001; Prata et al., 2009; Wirgenes et al., 2010); Oligodendrocyte Linage Transcription Factor 2 (Olig2) (Georgieva et al., 2006), Reelin (RELN) (Shifman et al., 2008; Liu et al., 2010), have also been associated with psychosis. In bipolar disorder, the consistency of candidate genes findings have been less as demonstrated in recent meta-analysis by Seifuddin et al. (2012).

Interestingly, although many of these genes were first identified as schizophrenia susceptibility genes, several studies have also shown an association with bipolar disorder, thus supporting suggestions that schizophrenia and bipolar disorder may share a common etiology (Bramon and Sham, 2001; Cardno et al., 2002; Murray et al., 2004; Craddock et al., 2006, 2009; Maier, 2006). Indeed, this is also supported by evidence that both schizophrenia and bipolar disorder share common underlying brain variations in white matter volumes (McDonald C., et al 2004). This would suggest that some genes and brain variations may be common to all psychotic disorders. It is therefore important to include all type of psychosis while investigating genetic influence on these illnesses.

1.13.5.3 *Genomewide association*

More recently, Genome Wide Association Studies (GWAS) have used a different approach. In candidate gene studies the loci of interest are chosen *a priori*, in contrast in GWAS the entire genome is interrogated in a hypothesis-free fashion by using panel of genetic markers that cover the entire genome. In this way GWAS enable the genome-wide study of the impact of common variants as defined for Minor Allele Frequency present by more than 5%

(MAF>5%). GWAS performed so far cannot explore the effect of rare variants (MAF<5%) that are not represented in the GWAS platform (Hardy et al., 2009).

In GWAS studies, this allele frequency of the genome-wide markers is compared between cases and controls. Genome-wide SNPs array commonly used include up to 1 millon SNPs. Additionally, GWAS focus on associations between single-nucleotide polymorphisms (SNPs) plus those proxy SNPs or markers in linkage disequilibrium (non-random associations of SNPs) and traits of illnesses such as psychosis in a systematic and more unbiased way. As in the candidate gene studies approach, if the allelic variant is more frequent in people with the illness, that specific allele is considered "associated" with the illness. The strength or effect size of the association is usually measured with Odds Ratio (OR) for “discrete trait” such as diagnosis or as proportion of variance explained for “quantitative trait” such as brain volume. Therefore, the identified SNPs are considered to mark a region of the human genome that increases the risk for the illness (Pearson and Manolio, 2008).

The advantages of the GWAS over candidate gene association studies lies in the possibility of looking at the entire genome at once rather than testing only one or few genetic regions. In addition, the quality control that these studies undergo is much more restrictive than candidate gene approach. For example population stratification can be adjusted using information from the genetic markers that enable the fine estimation of ethnic stratification. In this way, ancestral genetic heterogeneity can be better controlled for. However, GWAS approach brings the disadvantages of multiple testing with the risk of finding an association just by chance. For this reason the statistical threshold of significance is established at $5X10^{-8}$ and very large samples are needed to achieve enough statistical power (Hirschhorn and Daly 2005). Moreover,

given the need of such large samples, replication of findings is more difficult to obtain (Pearson and Manolio, 2008).

Therefore, larger samples achieved by international collaboration and more strict significant thresholds have been proposed by the Wellcome Trust Case Control Consortium to overcome these problems (WTCCC, Nature 2007). Although GWAS approach is not driven by a specific candidate gene, once the polymorphism is identified, it leads to studies to specify which genes are causal. Some previous GWAS studies looking at associations in psychosis had not consistently identified a genetic marker for these illnesses (WTCCC, 2007; Sullivan et al., 2008; O'Donovan et al., 2008; International Schizophrenia Consortium, 2009; Baum et al., 2008).

More recently, meta-analyses of Genome-wide association studies (GWAS) have shown the advantage of combining the results of independent studies to obtain a pooled effect estimate). Once a genome-wide association signals is performed the region is sequenced to identify the "causal" variant/variants and association is tested in the population. The meta-analysis carried out by Shi et al., found a significant association with schizophrenia in a region of linkage disequilibrium on chromosome 6p22.1 which includes genes related to immune system and chromatin modification gene (Shi et al., 2009). Additionally, other associations with genome-wide significance have been found with neurogranin (NRGN) that encodes a protein involved with the function of N-methyl-d-aspartate receptor (NMDA) and transcription factor 4 (TCF4) involved in brain development (Stefansson et al., 2009). It is worth mentioning that Stefansson et al. significant results are based on a combination of 3 European GWAS. Meta-analysis of GWAS in bipolar disorders have consistently reported association of the illness and chromosomal region 3q21 (Scott et al., 2009; McMahon et al., 2010; Vassos et al., 2012)

1.14 The issue of power analysis in genetic studies

The power of a statistical test represents the possibility that the study will be successful in detecting a true effect and is dependent on a number of factors, including the magnitude of the effect, the sample size and study design, and the specified false-positive rate. Power analysis is appropriate when the concern is with the correct rejection, or not, of a null hypothesis. In this way, if the study is well powered, a statistically significant result would correctly reject the null hypothesis (i.e. no differences between the comparison groups). Therefore, power calculations are usually carried out during the planning stages of a study, most typically in determining the sample size required.

There are mainly four parameters that affect the statistical significance of a test:

1- the effect size
2- the sample size (*N*)
3- the alpha significance criterion (*α*)
4- statistical power, or the chosen or implied beta (*β*)

Traditionally, for many small-scale studies (small N), investigators have, by convention, adopted values such as α = 0.05 and β = 0.20 (80% power) as representing a realistic and adequate trade-off. However, for larger studies (lager N), and more specifically in genetic analysis, the issue is less about determining a difference between samples but rather with the estimate of the effect size. The effect size is an estimated measurement of the strength of the relationship between variables in a sample. Although the effect size cannot make any claim about whether the apparent relationship in this particular sample reflects a true relationship in the population, the larger the effect size, the more likely that it is a more accurate representation of the population.

In order to increase the statistical significance of a test, the sample size can be clearly increased adding more subjects to the sample while the effect size can also be increased; for example, by reducing the measurement error with more

accurate phenotyping, or through genotyping a region of interest more densely.

With the increasing advantages in genetics technology, today's genetic studies carry the possibility of looking at the multiple genome SNPs at once rather than testing only one or few genetic regions. However, any statistical analysis involving multiple hypotheses carries the risk of the type I error (false positive) if appropriate measures are not taken. Therefore, measures such as applying a higher threshold of stringency to reject a hypothesis in order to compensate for the multiple comparisons being made (e.g. as in the Bonferroni method) should be used. In this situation, the power analysis should reflect the multiple testing approaches to be used. For this reason the statistical threshold of significance is established at $5X10^{-8}$ and very large samples are needed to achieve enough statistical power (Hirschhorn and Daly 2005) in genetic analysis.

1.15 Genes under unvestigation

As described so far in this chapter, there is the vast evidence of plausible genetic influence in brain abnormalities described in psychosis as well as genetic association between genes and psychotic illness. Therefore, in order to evaluate these factors in my thesis, I have carried out a literature review of candidate genes for brain volumes in psychosis to be tested. The selection process for the candidate genes is explained in chapter 2: Methods. In the next section I will describe the relevant genes that I have included in my thesis.

1.15.1 *SBNO1* and Hight Mobility Group AT hook-2 (HMGA2)

The latest GWAS studies have described new common variants that regulate brain size (Taal et al., 2012; Stain et al., 2012; Ikram et al., 2012) in healthy population. These reports came from two multinational consortia: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

and Enhancing Neuro Imaging Genetics Through Meta-Analysis (ENIGMA). Each consortium investigated its participants' genomes for single nucleotide polymorphisms (SNPs) associating with brain measures on magnetic resonance imaging (MRI) scans. For validation they then sent their top hits to the other consortium, and additional researchers. Although there were no SNPs associated with total brain volume a number of gene variants were associated with hippocampal size or intracranial volume (this was measures total space within the skull, regardless of brain size). These new findings offer an opportunity to explore these variances in relation to putative endophenotypes such as hypocampus and brain volumes in psychiatric diseases such as psychosis. *SBNO1* showed an effect on brain growth in early life (Taal et al., 2012) while *HMGA2 gene*, that encodes for protein that during development regulates stem cell renewal (Nishino et al., 2008), was associated with development of intracranial volumes in adults (Stein et al., 2012). These findings give a solid base for further investigations on the genetic effects on neurodevelopment in early and later life of psychiatric illnesses like psychosis. I have evaluated these genes in my thesis. To my knowledge, in my thesis is the first time that *SBNO1* and *HMGA2* genes are assessed in a sample of patients with psychosis.

1.15.2 *Catechol-O-Methyl Transferase (COMT)*

The gene for catechol-O-methyl transferase (*COMT*) codes for the enzyme catechol-O-methyl transferase involved in the synthesis and degradation of catecholamines. It is functionally polymorphic, with a variable amino acid, Val158Met. Some studies of the *COMT* gene have tested for association with the low activity (Met) allele (Akil et al 2003; Bray et al 2003) and psychiatric disorders (Shifman et al 2004; Hoth et al., 2006). Evidence suggests that the high activity (Val) allele, through increased catabolism of dopamine in the prefrontal cortex, may slightly increase the risk for schizophrenia and may explain some of the differences observed differences in cognitive performance and prefrontal cortical functioning between cases and controls (Egan et al.,

2001). Two previous meta-analyses testing for association between *COMT* and schizophrenia have provided contradictory results. One study showed association only in cases of European descent (Glatt et al., 2003). However, Fan et al (2005) meta-analysis did not confirm either association or the presence of ethnic differences (Fan et al., 2005). A large-scale European study involving patients with schizophrenia and controls and relatives tested positive markers and haplotypes identified in studies, but they found no significant association despite generous power (Williams et al., 2005). A study by Ohnishi and colleagues (2006) has shown that individuals homozygous for the Val-*COMT* allele have significant reduction of volumes in the left anterior cingulate cortex (ACC) and the right middle temporal gyrus (MTG) compared to Met-*COMT* carriers. In addition, significant genotype-diagnosis interaction effects on brain morphology were noted in the left ACC, the left parahippocampal gyrus and the left amygdala-uncus. Interestingly, genotype effect on brain morphology was not significant in the control group. Schizophrenics homozygous for the Val-*COMT* showed a significant reduction of volumes in the bilateral ACC, left amygdala-uncus, right MTG and left thalamus compared to Met-*COMT* schizophrenics. These findings suggest that the Val158Met polymorphism of the *COMT* gene might contribute to morphological abnormalities in schizophrenia (Ohnishi T. et al, 2006).

1.15.3 *Brain-derived neurotophic factor (BDNF) and BDNF receptor neurotrophic tyrosine kinase receptor type 2 (NTRK2)*

The BDNF/NTRK2 signaling pathway plays a critical role in regulating the survival and differentiation of neuronal populations. Moreover, this complex is also implicated during synaptic transmission (Huang and Reichardt, 2001) and neuroplasticity (Lu, 2003). *BDNF* is a trophic protein involved in neurodevelopment and also in modulating activity-dependent synaptic plasticity among mature neurons arborization (Binder and Scharfman, 2004; Angelucci et al., 2005). Furthermore, it decreases dendritic growth, particularly in the hippocampus and neocortex (Gorski et al.,2003; Lu et al.,2003). The

single nucleotide polymorphism (rs6265), valine (Val) to methionine (Met) substitution at codon 66 in the pro*BDNF* protein produces, inefficient *BDNF* trafficking, reduced activity-dependent *BDNF* release, and poorer hippocampus-mediated memory (Egan et al.,2003; Dempster et al.,2005; Tan et al.,2005). Additionally, the *BDNF*-Met variant has been associated in healthy volunteers with a decrease in volume of hippocampal and frontal lobe (Pezawas et al., 2004; Bueller et al., 2006). There are some theories that have been put forward to explain in which ways BDNF-Met variant may decrease gray matter volumes. For example, it has been suggested that in a relatively *BDNF*-deficient environment during embryogenesis, fewer neurons survive, and surviving neurons have smaller soma size diminishing dendritic growth (Gorski et al.,2003). These findings suggest the need of *BDNF* for their normal development. Another theory is that the functional complex of the *BDNF*-Met variant and its receptor (*NTRK2)* might influence gray matter volumes beyond neurodevelopment through modulating neuroplasticity in mature neurons (Xu et al, 2000). In psychosis studies, there is evidence for an increased risk for psychosis with the Val-Met (66) polymorphism (Neves-Pereira et al., 2002, 2005; Rosa et al., 2006). Moreover, in schizophrenia, previous cross-sectional MRI studies have found that Met allele carriers with schizophrenia showed smaller brain volume in areas such as the frontal and temporal gray matter (Ho et al., 2006), hippocampus (Szeszko et al., 2005) and temporal grey matter (Ho et al., 2006).

1.15.4 *Neuregulin (NRG1)*

NRG1 is part of the neuregulin family of factors involved in growth and in neural migration and axon guidance (Mei and Xiong, 2008). Therefore, *NRG1* has become a strong candidate gene when looking for genetic and endohenotype associations in psychosis. Furthermore, studies in childhood onset schizophrenia have described a crucial role of NRG1 SNP8NRG221533 in the grey and white matter volume changes observed in carriers of the risk allele as well as for other *NRG1* SNPs (Addington et al., 2007). This evidence

further supports the role of *NRG1* as one of the candidates for the neurodevelopmental theory of psychosis.

Some candidate genes are argued to regulate proliferation, migration and differentiation during the neurodevelopmental process including Neuregulin (*NRG1*) maps in chromosome 8q12. Neuregulin was firstly described in a genome-wide linkage scan of Icelandic families (Stefansson et al., 2002). The risk haplotypes are described as sharing a "common core haplotype" (HapICE). This consists of five SNPs (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177 and SNP8NRG433E1006) and two microsatellites (478B14-848 and 420M9-1395). For NRG1, SNP8NRG243177 T allele (Stefansson et al., 2002; Rousos et al., 2010) and SNP8NRG241930 G allele (Stefansson et al., 2002) and in Iranian populations (Sheriati et al., 2011) have been described as the risk alleles for schizophrenia. In both polymorphisms T allele has been shown to be the minor allele frequency.

The HapICE association has been replicated in other populations such as Scottish (Stefansson et al., 2003), Irish/UK (Williams et al., 2003), Japanese (Iwata et al., 2004), Japanese individuals (Fukui et al., 2006) and Chinese (Tang et al., 2004; Li et al., 2004). However, other studies in Causcasian population (Thieselton et al.; 2004, Duan et al., 2005; Ingason et al., 2006; Rosa et al., 2007) have not replicated this association and no susceptibility variant has yet been identified. Replication studies looking at specific polymorphisms have not always been consistent with the original association studies. For instance, with SNP8NRG221533, Stefansson et al (2002) reported the A allele as the risk allele for schizophrenia. However, other studies have found the T allele to be the risk allele in adults samples (Bakker et al., 2004) as well as in childhood onset schizophrenia (Addington et al., 2007). These inconsistencies might be related in ethnic heterogeneity (Gardner et al., 2006) and to the genetic heterogeneity of the psychotic disorders.

SNP8NRG241930 G alleles and SNP8NRG243177 T allele has been found in association with other endophenotypes like slower prepulse inhibitions in patients with schizophrenia (Rousos et al., 2010). Additionally SNP8NRG243177 T allele has also been associated with brain deviations in healthy volunteers (McIntosh, et al., 2008; Barnes et al., 2012), in patients with psychosis (Mata et al., 2009) and abnormal function in functional neuroimagings in individuals at high risk for schizophrenia (Hall et al., 2006). These findings support the notion of a possible role for these polymorphisms in the structural brain changes associated with schizophrenia.

1.15.5 *Dysbindin (DTNBP1)*

DTNBP1, otherwise known as dysbindin, binds to α- and β-dystrobrevin in muscle and brain. It is present in muscles and axon terminals in the cerebellum and hippocampus of the adult mouse brain (Benson et al., 2001). A plausible function in glutaminergic synapses has been described by Benson et al. (Benson et al., 2001). Glutamate 2/3 (mGlu2/3) receptor agonistic drug was the first drug developed not acting as a dopamine antagonist based on the glutamatergic hypothesis of schizophrenia (Patil et al., 2007; Chaki and Hikich, 2011). *DTNBP1* mRNA is widespread in the brain. Reduced mRNA expression in dorsolateral prefrontal cortex has been described in patients with schizophrenia in comparison to healthy controls (Weickert et al., 2004). Moreover, this is also true for the hippocampal formation in patients with schizophrenia, who have been reported to show reduced protein expression in the brain (Weickert et al., 2008). Reduced *DTNBP1* mRNA expression in cerebral cortex has also been associated with risk haplotypes for schizophrenia (Bray et al., 2005).

Dysbindin was firstly described as associated with schizophrenia in linkage studies, which identified the 6p24-p22 as a region of high schizophrenia susceptibility (Moises et al., 1995; Straub et al., 1995; Wang et al., 1995).

Later investigations showed a genetic association for a susceptibility locus on chromosome 6p22.3 in Irish pedigrees with several single nucleotide polymorphisms (SNPs) (Straub et al., 2002). Associations with six of these polymorphisms were replicated in sib-pair and triad families (Schwab et al., 2003). Association analysis of the 6p22.3 region has shown strong association with schizophrenia in Caucasian, Hispanic (Funke et al., 2004) and Chinese populations (Tang et al., 2003). However, there is conflicting evidence for an association with schizophrenia, with some studies failing to identify an association of the originally described *DTNBP1* SNPs and schizophrenia (Li et al., 2005; Datta et al., 2007; Morris et al., 2003; Peters et al., 2008; Sanders et al., 2008). A meta-analysis identified a weak association of a *DTNBP1* SNP with schizophrenia, which was not significant after multiple testing (Li et al., 2007). Despite failing to replicate the original SNPs described in the Scottish population, Li et al. (2005) showed a significant association between *DTNBP1* P1320 and P1757 and schizophrenia in the Chinese one. This provides support for *DTNBP1* as a susceptibility gene for schizophrenia in the Chinese population, albeit with haplotypes different from those of the original study.

Finally, in order to understand possible causal factors for the reported differences in brain volumes in psychosis, some studies have investigated genes involved in normal brain development such as Reelin (*RELN*), Microcephaline (*MCPH, ASPM*) and Oligodendrocyte lineage transcription factor 2 (*OLIG2*) in patients with psychosis. In this study, I have also explored the genetic influence of several neurodevelopmental genes that have been described as candidate gene to influence brain volume in patients with psychosis and in healthy populations.

1.15.6 *Microcephalin (MCPH) and (ASPM)*

Human brain volume is correlated with general intelligence, working memory, perceptual organization and processing speed (Postuma et al., 2003;

McDaniel 2005). Studies looking at possible genes modulating brain size have recently focused their attention on primary microcephaly. This condition is inherited as an autosomal recessive trait defined by no other significant neurological deficits than mild-to-moderate mental retardation. Subjects with primary microcephaly, show a well preserved gyral pattern, with no major abnormality in cortical architecture despite this marked reduction in size (Mochida and Walsh 2001; Bond et al., 2002). In situ hybridization demonstrates that microcephalin is expressed in particular around the lateral ventricles of the developing forebrain, suggesting that the gene has a role in regulating the size of the cerebral cortex (Jackson et al 2002).

Jackson et al (1998) mapped the candidate region for the MCPH1 in chromosome 8p22. Subsequently, four more loci for primary microcephaly have been identified: MCPH2 on chromosome 19q13×1–13×2 (Roberts et al., 1999) and MCPH3 on chromosome 9q34 (Moynihan et al 2000) mapped in families from the northern region of Pakistan; MCPH4 on chromosome 15q (Jamieson et al 1999) in families from Morocco; and MCPH5 on chromosome 1q25–q32 mapped in families from Turkey (Jamieson et al., 2000) and Multan in Pakistan (Pattison et al., 2000). To date, there are four genes have been reported as responsible for primary microcephaly, these include microcephalin (MCPH1, MIM 607117), abnormal spindle-like microcephaly-associated (ASPM=MCPH5, MIM 605481), cyclin-dependent kinase 5 regulatory associated protein 2 (CDK5RAP2=MCPH3, MIM 608201) and centromere-associated protein J (CENPJ=MCPH6, MIM 609279) (Bond et al., 2002, 2005; Jackson et al., 2002). Of these, the ASPM=MCPH5 mutation has been described to account for about 43% of the total autonomic recessive primary microcephaly (Roberts et al., 1999; Bond et al., 2003).

Studies looking at the influence of MCPH genes influence on brain volume outside primary microcephaly have shown contradictory results. While some have found positive associations of the homozygous males containing the

derived alleles of rs1057090 with larger cranial volumes in Chinese population (Wang et al 2008), others have not found an association between brain volume and MCPH and ASPM (Woods et al., 2006; Dobson et al., 2007).

As in primary microcephaly, patients with schizophrenia show a well preserved gyral pattern, with no major abnormality in cortical architecture but a small reduction in brain size when compared to healthy controls (Wright et al., 2000). Additionally, general intelligence, working memory, perceptual organization and processing speed, shown to be affected in primary microcephaly have also been extensively reported in patients with psychosis (Kalkstein et al., 2010). Therefore, this gene has been studied in patients with schizophrenia, although so far with negative results (Rivero et al., 2006). Despite these initial negative results, genes linked to this condition can offer potential insights into the development and evolution of the cerebral cortex in patients with psychosis.

1.15.7 *Oligodendrocyte lineage transcription factor 2 (OLIG2)*

Oligodendrocyte lineage transcription factor 2 (OLIG2) maps to the chromosome 21q22.11. It is expressed throughout the brain during brain development and adulthood, and it is also involved in the formation of glial scar after brain injury (Ono et al 2009). The main function is that of a transcription factor that regulates the differentiation of motor neurones and oligodendrocytes (Ono et al 2009). Genes associated to myelination and oligodendrocyte functions have been associated with schizophrenia (Gerogieva et al 2006). OLIG2 expression has been shown to be reduced in patients with schizophrenia (Gerogieva et al 2006; Mitkus et al., 2008). Particularly, the OLIG2 SNP rs10590004 allele A has been associated to schizophrenia (Gerogieva et al 2006), and seems to be associated with altered white matter integrity in healthy volunteers (Prata et al., 2012) and in patients with obsessive-compulsive disorder with psychotic symptoms (Stewart et al., 2007). Interestingly, other polymorphisms have been

associated with psychosis in patients with Alzheimer's disease (Sims et al. 2009). So far, no study has investigated OLIG2 in affective psychoses.

1.15.8 *Reelin (RELN),*

RELN gene encodes the Reelin protein encoded. It was first described as the gene mutated in mice with an anomalous reeling gait (De Arcangelo et al., 1996). Reelin is an extracellular matrix-associated glycoprotein expressed in the developing brain. It has been described to be involved in neuronal migration and synaptic plasticity, learning and memory (Beffert et al., 2005) and mostly present in GABAergic neurons (Pesold et al., 1998). Given its involvement in the brain development, it has been a gene of interest in relation to psychosis. Studies of postmortem brain tissues from patients with schizophrenia have shown a reduction of RELN mRNA and Reelin up to 50% (Impagnatiello et al., 1998, Eastwood et al., 2003 and 2006). Although reductions of RELN mRNA in brain tissue have been described in different regions of the brain using diverse techniques, they have been one of the most consistent molecular findings in RELN and psychosis (Knable et al., 2001). Nonetheless, a study evaluating RELN mRNA in brain tissue by Oviada and Shifman (2011) failed to replicate this reduction in schizophrenia samples. However, the authors described a significant reduction of the amount of the short RELN isoform in bipolar disorder patients.

A genome-wide association study in schizophrenia in an Ashkenazi Jewish (AJ) population has revealed a sex-specific association between intronic single nucleotide polymorphism (SNP) in the reelin RELN gene and schizophrenia (Shifman et al., 2008). In addition, RELN SNP (rs7341475) was also shown to have a female-specific association (Shifman et al., 2008, Liu et al. 2010). Moreover, a meta-analysis evaluating four GWAS conducted in schizophrenia and the excluding sample described by Shifman et al (2008) confirmed the association with rs7341475 in women with schizophrenia with a calculated odds ratio of 1.11 (Ben-David et al., 2010). Despite the described

associations, when looking at intermediate phenotype such as measures of brain structure, brain function, and gene expression; some studies did not find a significant association with rs7341475 genotypes (Tost et al., 2010). However, other studies have reported association between working memory and other regions of RELN gene in patients with schizophrenia (Wedenoja et al., 2009). Interestingly, association of Reelin gene and patients with bipolar disorder has also been found (Fatemi et al., 2000; Guidotti et al., 2000). More recently, RELN SNP rs362719 was described to be associated with risk to bipolar disorder in women (Goes et al., 2010). These findings continue to support the hypothesis that RELN is implicated in the pathway of the development of psychosis.

1.15.9 *Corticotropin-Releasing Hormone Receptor 1 (CRHR1)*

CRHR1 has been associated in animal models with brain development in young mice (Hsuchou et al., 2010). Additionally, Corticotrophin-releasing factor (CRF) is a stress hormone that acts as a neuromodulator binding to CRHR1 in the hypothalamic-pituitary-adrenocortical (HPA) axis, playing an important role in the development of major depression (Arborelius et al., 1999; Hsu et al., 2012). Postmortem studies have shown decreased levels of *CRHR1* mRNA in the frontal cortex in patients with major depression, suggesting that *CRHR1* plays a role in the pathophysiology of the disorder (Nemeroff et al., 1988; Merali et al., 2004). In addition, it has been argued that corticotrophin-release hormone, released during stress, might change the synaptic spines stability leading to brain cortical malfunctioning and involved in the development of depression and psychoses (Bennett, 2008). A recent GWAS study described an association between *CRHR1* and brain volume in healthy population (Ikram et al., 2012). These findings combined with previous reports in human studies describing elevated levels of CRF in the cerebrospinal fluid of patients with post-traumatic stress disorder with psychotic symptoms (Sautter et al., 2003) make CRHR1 gene a suitable candidate gene for brain volume changes in psychosis.

1.16 Conclusion

Neuroimaging studies give a good insight into the neurodevelopmental theory of psychosis. Structural MRI studies have consistently shown the presence of reductions of grey matter volumes and of ventricular enlargement in individuals with psychosis. Furthermore, longitudinal studies on early onset schizophrenia suggest the presence of an exaggeration of the normal cortical development in affected individuals. Finally, evidence that relatives of individuals with psychosis may share some of these brain alterations implies a role for an underlying genetic vulnerability to these alterations. These findings suggest that during the adolescent and early adulthood there may be a combination of factors such as genetic loading, brain maturation and environmental factors that take place and potentially underlie the onset of psychosis. In this study, I will investigate the relationship between genes that have been proposed as associated with neurodevelopment and psychosis, and their influence on global brain volume deviations. I will achieve this by comparing a large sample of patients with psychosis, their unaffected relatives and healthy controls.

Chapter 2: Aims and general methodology

2.1 Aims of the thesis

One of the difficulties with genetic and neuroimaging analyses is that large numbers of subjects are needed to test specific hypotheses.

In this Thesis, I aimed to examine the role of recently identified susceptibility genes for psychosis and of genes involved in neurodevelopment in influencing brain volumes in subjects with psychosis, their relatives and in healthy controls. In order to achieve this, I have proceeded with two main steps:

-First, I conducted a calibration study to explore whether MRI scans acquired with different neuroimaging protocols used in 3 different studies, the Aetiology and Ethnicity in Schizophrenia and Other Psychosis (AESOP) study, The Maudsley Family study and The Maudsley Twin study, could be combined to reliably estimate global brain volumes (grey matter, white matter, and whole brain volumes). These studies all acquired a three-dimensional Fourier-transformed spoiled-gradient-recalled acquisition in the steady state (SPGR), with the same GE Sigma 1.5-T system (GE Medical Systems, Milwaukee), at the Maudsley Hospital. This first step was conducted in a sample of 12 healthy individuals, each undergoing an MRI scan with the different protocols.

-Second, I identified, gathered, genotyped where necessary (i.e if new samples were collected), and analyzed subjects in whom both DNA and MRI structural brain data were available from the above studies (AESOP, Maudsley Family study, Maudsley Twin study). Subjects included: individuals with psychosis, their relatives, and healthy individuals.

2.2 Hypotheses to be tested

I tested the following hypotheses:

2.2.1 *Brain volumes (MRI analysis):*

a. Smaller grey, white and total matter volumes would be present in subjects with psychoses compared to their relatives and to healthy controls; and

b. These volume differences would be more marked in patients with schizophrenia than in bipolar patients in comparison to healthy volunteers;

c. The relatives of patients with psychosis will show volumetric brain changes intermediate between those of patients and healthy individuals;

2.2.2 *Genetic analysis:*

My analysis of the genetic data was restricted to the genes already genotyped for the three different studies. Among those, I chose to focus my attention in exploring genes that have been described as:

- Candidate genes for psychosis such as: *COMT, BDNF, NRG1* and *DTNBP1*. I predicted that carriers of *COMT* (rs4680)-val allele, *BDNF (*rs6265)-Met allele and certain polymorphisms of *NRG1* (SNP8NRG241930, SNP8NRG221533, rs6994992), *DTNBP1 (*rs1047631, rs875462), *RELN* (rs7341475) would be associated with smaller grey matter volume in the risk allele carriers

- Genes that have been described to influence brain volume in normal populations but have not been explored in psychosis: I hypothesized that specific polymorphysms of *MCPH* (rs2305022, rs930557, rs1057090, rs2912016, rs3762271), *SBNO1* (rs7980687), *HMGA2* (rs1042725) and *CRHR1* (rs11655470) would be associated with show smaller total brain volume in the risk allele carriers

- Genes that have been described to influence white matter structure, such as *OLIG2* (rs1059004), would be associated with smaller white matter volumes in the risk allele carriers

2.3 Study samples

Subjects included in this Thesis were recruited and evaluated as part of 3 large studies that have been conducted within the Institute of Psychiatry, King's College London. These are: the Aetiology and Ethnicity in Schizophrenia and Other Psychosis (AESOP) study, The Maudsley Family study and The Maudsley Twin study. These were all approved by the London and Maudsley NHS Trust and Institute of Psychiatry Research Ethics Committee. All subjects included in the studies gave their informed consent to participate. In the following sections I will describe the characteristics of each study in turn, and specify the respective inclusion and exclusion criteria.

2.3.1 *The Aetiology and Ethnicity in Schizophrenia and other Psychosis (AESOP) study*

The Aetiology and Ethnicity in Schizophrenia and other Psychosis (AESOP) study was an incidence study of functional psychosis carried out over a two-year period, between 1st September 1997 and the 31st August 1999. A sample of first episode psychosis patients was recruited from secondary psychiatric in-patient and out-patient services of the South London and Maudsley NHS Trust, serving a large area of South London encompassing the boroughs of Lambeth, Southwark and Croydon. Cases were recruited from the at risk population that included all people between 16 and 64 years old, presenting to psychiatric services with first-in-lifetime functional psychosis.

In addition, a sample of healthy individuals was recruited from the same catchment area. The sample of control subjects was selected randomly from the population, using the same sampling frame as that used by the Office of Population and Census Statistics (OPCS) Psychiatric Morbidity Survey, namely the postal address file (PAF) (Jenkins R. et al, 1995). This method was chosen to ensure comparability between cases and controls. With this approach, a random sample of ten target addresses was generated for each

case for which controls were recruited. Subjects selected were contacted three times (morning, afternoon, evening) to find an eligible control subject aged between 16 and 65 years willing to participate. This method broadly matched cases and controls by area of residence.

Inclusion and exclusion criteria

Patients with psychosis identified for the study were first screened for the presence of psychotic symptoms with the “Screening Schedule for Psychosis” based on the Psychosis Screening Questionnaire (Appendix 1) (Bebbington and Nayani 1995). This questionnaire explores the presence of a broad range of psychotic experiences. Subjects positive for the questionnaire who gave written consent were assessed with a diagnostic tool, the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.0) (WHO, 1992a). The SCAN is a tool designed to enable the formulation of psychiatric diagnosis (Janca at al, 1994). Using the data obtained with the SCAN, consensus diagnoses were agreed for all subjects. Those subjects who did not meet ICD-10 (1992) criteria for a diagnosis of functional psychosis were excluded from the study. The cases identified were the entered into further assessments if they satisfied the following inclusion criteria:

-Age 16-64 years

-Resident within the area of the study

-Absence of moderate or severe learning disability as defined by the ICD-10 (WHO, 1992b)

-Presence of a functional psychosis (ICD-10 F10-19, excluding coding F1x.0 for acute intoxication; F20-29 and F30-39, psychotic codings).

-No previous contact with psychiatric services for psychotic symptoms.

-Agreement to undergo an MRI scan and provide DNA sample.

Healthy individuals were included if they satisfied the following inclusion criteria:

- Age 16-64 years

- Resident within the catchment area of the study
- Absence of positive scores in the PSQ
- Absence of moderate or severe learning disability as defined by the ICD-10 (WHO, 1992b)
- Agreement to undergo an MRI scan and provide DNA sample.

In addition, both patients and healthy controls were excluded if they had:
- History of neurological disorder or head trauma resulting in loss of consciousness for more than 1 hour
- Poor English language skills
- History of psychotic symptoms in the past.

2.3.2 *The Maudsley Family study*

The Maudsley Family Study is a large study that started in 1992 with the aim to investigate genetic liability to schizophrenia and bipolar disorder with psychotic symptoms and proceeded with several phases of recruitment. The recruitment of the sample I included in my Thesis was carried out over a period of 6 years (1997 to 2003). The families with at least one member affected by psychosis were invited to participate in the study. For schizophrenia, the study increased the variation of probable genetic liability by recruiting families who were either “familial”, where the index patient had other first- and/or second-degree relatives affected with a psychotic disorder, or “non-familial,” where the index patient had no known relatives with a psychotic disorder. For bipolar disorder, all subjects included in the study had a family history of bipolar disorder or another functional psychosis in the first or second degree relatives. Families were recruited by voluntary support groups such as the National Schizophrenia Fellowship (Rethink) and the Manic Depressive Fellowship. Additionally, direct referrals by the patient’s treating physician were made to the research team. The majority of the families recruited lived in southeast England.

A group of control subjects was recruited from the community through advertisements in local newspapers or from staff, and were group-matched by age, gender, social classes, level of education and handedness. None of the control subjects had a life-time or family history of psychosis, and no relatives had a life-time history of psychosis.

Inclusion and exclusion criteria

For the whole sample, the inclusion criteria were:

- Age 16–69 years
- First language English
-Agreement to undergo an MRI scan and provide a DNA sample.

Exclusion criteria were:

- Current or previous organic brain disease, history of head trauma resulting in loss of consciousness for more than 5 minutes
-Fulfilled criteria for DSM-IV substance or alcohol dependence in the 12 months before the assessment.

2.3.3 *Maudsley Twin study*

The Maudsley Twin study aimed to investigate the role of genetic liability to schizophrenia and consisted of pairs of monozygotic (MZ) twins concordant for schizophrenia or schizoaffective disorder, and pairs of MZ and dizygotic (DZ) twins discordant for schizophrenia; in this group the co-twin was free of any psychotic illness. The patients were referred by the responsible psychiatrist from the whole of the UK. The study also included a control group consisting of pairs of MZ and DZ twins. This group was recruited from the Institute of Psychiatry Volunteer Twin Register and also with the help of advertisements in the media.

The subjects in the schizophrenia group were clinically stable at the time of assessment and had undergone no recent changes to their medication.

Inclusion criteria

- Aged 16 to 65 years old
- Patients fulfilled criteria for a diagnosis of schizophrenia in all the sub-types according to DSM-IV
- Healthy volunteers had not had personal or family history up to second degree relative, of a psychotic or schizophrenia spectrum disorder.

Exclusion criteria

- History of neurologic complications or severe head injury with loss of consciousness.
- Fulfilled criteria for substances misuse at the time of assessment based on DSM-IV.

2.4 Socio demographic and Clinical assessments

The same socio-demographic variables were evaluated across the 3 samples. In the AESOP study, general demographic information were obtained for the patients with a schedule specifically designed for the study were they self-reported their date and place of birth, self-asserted ethnicity, years of education and employment. For the Family and Twin Studies socio-economic status was based on details of parental occupation at birth and derived from the Office of Population Censuses and Surveys Standard Occupational Classification (office of population censuses and surveys, 1991).

2.4.1 *Handedness*

This was assessed with the Annett scale (Annett 1970). This scale assesses the subject hand preferences for 12 actions (appendix 2). Based on these preferences subjects are classified into right, left or mixed handedness.

2.4.2 *Cognitive function*

In the AESOP and Maudsley Family Study IQ was estimated with the Wechsler Adult Intelligence Scale – Revised (WAIS–R) (Wechsler, 1981). The

Maudsley Twin Study used Wechsler Adult Intelligence Scale-III (Wechsler, 1997).

2.4.3 *Family history of mental disorders*

Across all studies the Family and Genetic Questionnaire for Genetic Studies (FIGS) (appendix 3) was used to gather information about family history of psychiatric disorders. The questionnaire is based on a structured interview that assesses the presence of psychiatric symptoms and illnesses in relatives.

2.4.4 *Clinical assessment of affected subjects*

Structural diagnostic interviews were performed in the 3 studies. Diagnostic interviews were complemented by medical notes, and collateral history from relatives or informants to confirm diagnosis.

In the AESOP study, subjects were interviewed with the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) carried out by trained raters. When a SCAN interview was not completed, symptoms information was entered in the Item Group Checklist (IGC) section of the SCAN alongside the case notes and informants' information. Consensus diagnoses were then reached according to the criteria of both the DSM-IV and the ICD-10 for a functional psychosis. As part of this study, inter-rater reliability for diagnosis was estimated for 20 cases and was judged satisfactory, with kappa scores ranging between 0.63 and 0.75.

The Maudsley Family and Twin studies used the Schedule for Affective Disorder and Schizophrenia - Lifetime Version (SADS-L) by Endicott J, Spitzer RL (1978). This questionnaire assesses and rates subject's symptoms and level of functioning in order to make a diagnosis compatible with Diagnostic and Statistical Manual of mental disorders 4th edition (DSM-IV) criteria (American Psychiatric Association. DSM-IV, 1994). In the Maudsley Family Study diagnoses were made by two independent senior psychiatrists, blind to

family structures, who made a consensus diagnosis to give best-estimate lifetime diagnoses according to DSM-IV criteria. As for the Maudsley Twin study, patients were diagnosed by one independent psychiatrist to improve consistency, blind to family structures, who made a diagnosis to give best-estimate lifetime diagnoses according to DSM-IV criteria.

2.4.5 *Exposure to antipsychotics*

Data on type, dose and length of exposure to antipsychotic drugs were recorded at the time of the MRI scan across all studies. This information was used to compute the total number of days of exposure to antipsychotic treatment up to the time of the MRI scan.

2.4.6 *Assessments of healthy volunteers*

The socio-demographic characteristics of healthy controls were obtained as those of patients in each study. In addition, the Psychosis Screening Questionnaire (Appendix 2) was used across all studies to exclude the presence of a psychotic disorder.

2.4.7 *Neuroimaging evaluation*

Images were processed using a Sun workstation (Sun Microsystems, Mountain View, USA) on a MAT-LAB (MathWorks, Natick, MA) platform, using Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm/software/spm2), version SPM8.

2.5 Neuroimaging

2.5.1 *MRI acquisition protocols*

All three studies acquired a three-dimensional Fourier-transformed spoiled-gradient-recalled acquisition in the steady state (SPGR), with the same GE Signa 1.5-T system (GE Medical Systems, Milwaukee), at the Maudsley

Hospital. All the images at the Institute of psychiatry were obtained in UNC format and stored in a Unix account.

The acquisition parameters in the AESOP study were: 1.5-mm-thick contiguous coronal T1-weighted MRI images extending through the entire brain acquired with TE = 5msec, TR = 13.8msec, TI= 450 msec, field-of-view = 20cm, acquisition matrix=256×256×123 and Flip-angle = 20^{o}.

From the Maudsley Family I included the MRI scans obtained in the period between 1999 and January 2002. The acquisition parameters were: 1.5-mm-thick contiguous coronal T1-weighted MRI images extending through the entire brain was acquired with TE=5.8 msec, TR=13.1 msec, TI= 450 msec, number of excitations=1, flip angle=20°, acquisition matrix=256×256×124.

The Maudsley Twin study used a number of neuroimaging protocols along the years. The study has collected MRI scans using the same SPGR sequence in 2 different scanners: a 1.5T GE scanner at the Maudsley Hospital and a 1.5T scanner at St Georges Hospital (Owens et al., 2012). In some cases, the same subject was scanned in both scanners. For compatibility reasons detailed below, in the Section on the calibration study (Chapter 2), I included in my study only images that were obtained in the 1.5T GE scanner based at the Maudsley Hospital. This is in fact where images for the other two studies included in this Thesis were also acquired. These images had identical acquisition parameters to those of the Maudsley Family study. For these reasons, thereon I will refer to this protocol as the “Maudsley Family-Twin protocol”. These acquisition parameters are described in Table 1. I did not include MRIs obtained at St George’s Hospital as they proved to be markedly different from those of the other 2 studies.

Table 2.1: Protocols acquisition variables

Protocol as original name	FAMSCHIS	TWINSCHIS long	TWINSCHIS Short = Family-Twin	AESSOPC
Name for calibration	3_F	7_T	3_F	4_A
Orientation	Coronal	Coronal	Coronal	Coronal
TR	13.1	35	13.1	13.8
TE	5.8	5	5.8	5
TI	450	0	450	450
Flip angle	20	35	20	20
xdim	0.859375	0.78125	0.859375	0.9375
ydim	0.859375	0.78125	0.859375	0.9375
zdim	1.5	1.5	1.5	1.5

2.5.2 *MRI processing*

Images from each study had been stored in separate Unix accounts at the Institute of Psychiatry. I first identified the correct MRIs for my project, ensuring, using an ad-hoc script, that only those with the exact scanning acquisition parameters were identified. Once identified, images were stored in a personal Unix account for pre-processing and analysis.

As a first step, all images were converted to UNC format, allowing for the application of an algorithm that applies intensity correction (see below).

All images then underwent quality control. This was done visually by me on all images to increase consistency. Quality control aimed to ensure full brain coverage, identification of wrap around artifacts, motion artifacts, presence of

intensity inhomogeneities, adequate grey/white matter contrast throughout the images, and identification of gross abnormalities.

2.5.2.1 *Intensity non-uniformity correction*

Intensity non-uniformity correction was applied to all MRI images to ensure optimal segmentation of the data into the correct tissue compartments. Magnetic resonance signal intensity from homogenous tissue should be uniform. However, due to artifacts introduced by a variety of sources which can lead to variations in signal intensity across the MRI image, this is hardly ever the case. There are in fact a number of reasons why this intensity non-uniformity may occur, such as differences in the sensitivity of the scanner reception coil, induced eddy currents, and non-uniform excitation (Sled et al 1998). Attention to such artifacts is particularly important in multi-study investigations, as changes in scanner signal may occur between serial time-points. An iterative non-parametric method for intensity non-uniformity correction on MRI volumes (N3) was applied prior to the segmentation stage of processing (Sled et al 1998). The application of the N3 was fully automated and applied to all images. This method has been shown to improve the sensitivity of voxel based morphometry (VBM) methods of analysis, if used prior to the SPM analysis (Acosta-Cabronera et al. 2008). The N3 algorithm was applied using a specific script. This script corrects intensity non-uniformities and also converts the images from coronal to axial orientation, and also to UNC to analyze format. Therefore, all the images obtained after this step are ready to be processed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8).

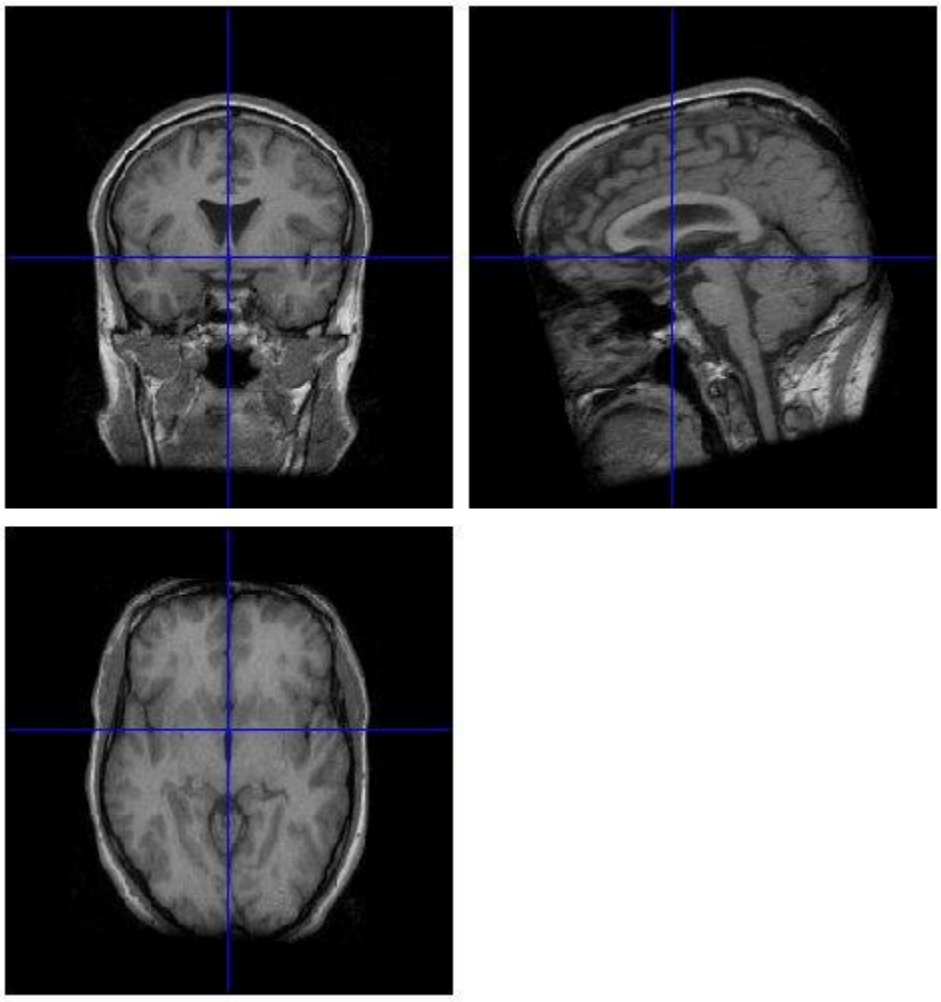
2.5.2.2 *Segmentation*

To increase the accuracy of the segmentation, I realigned all the images to an image that I selected as a good quality image. As changes in scanner signal will occur between serial time-points, I selected for this purpose an image collected in the year 2001. This year was chosen as it was the mid-point in

time when the MRI for the 3 studies were obtained (between 1997 and 2003). I manually reoriented the selected image along the AC-PC line using the key landmarks of the Talairach and Tournoux atlas (1988). In their description, the midpoint of the anterior commissure (AC) is located first, serving as the origin of Talairach space. The brain is then rotated around the new origin (AC) so that the posterior commissure (PC) appears in the same axial plane as the anterior commissure. The connection of AC and PC in the middle of the brain forms the y-axis of the Talairach coordinate system. The x-axis runs from the left to the right hemisphere through AC. The z-axis runs from the inferior part of the brain to the superior part through AC. In order to further specify the x and z-axes, a y-z plane is rotated around the y (AC-PC) axis until it separates the left and right hemisphere (mid-sagittal plane). The reorientation was done using the reorientation tool of SPM, where the image can be reoriented by manually manipulating the parameters along the 3 main axes. Once I was satisfied with this re-orientation, I saved this to be used as the representative scan (Figure 1). All the images were then realigned against this representative scan. From this point on, the realignment was a fully automated process that is applied with SPM. This method ensures that all images are realigned and corrected to the same parameters as the “representative scan” before segmentation.

SPM8 before segmentation, the images undergo spatial normalization to the same stereotactic space by registering them to the same template image. In this way SPM8 spatially normalizes the images by matching and estimating each image to the optimum 12 parameters affine transformation (Ashburner et al., 1997). This is done within a Bayesian framework whereby the Maximum a Posteriori (MAP) estimates the spatial transformation using a priori knowledge of normal variability of brain size. After this process, the affined transformed images are corrected for non-linear shape differences (from the template) by a linear combination of smooth spatial basis functions (Ashburne and Friston 1999).

Figure 2.1 Image re-orientation using the key landmarks of the Talairach and Tournoux atlas. This image was used as the representative scan.



The realigned and spatially normalized images were then used for tissue classification and segmentation. For the segmentation, SPM uses a mixed model cluster analysis to identify voxel intensities matching grey, white tissue and Cerebrospinal Fluid (CSF), using a priori knowledge of these tissue distributions from probability maps. As the segmentation step done with SPM also includes correction of intensities (Ashburner and Friston, 2000); in order to avoid duplicating the correction of intensity when applied the N3 algorithm to the images, the parameters were set as: for the “bias regularization”: extremely heavy regularization (10) and for the “Bias FWHM”: 150mm cut off as recommended in the SPM manual. The remaining parameters were the SPM8 default ones.

From the probability maps obtained, volumes of grey and white matters and Whole Brain Volume (WBV) were calculated in both voxels and litres. WBV was calculated summing the grey and white matter volumes.

2.5.2.3 *Volume extraction*

The volumes were obtained by using a script specifically designed for this purpose. This script runs in the pre-processed and segmented images. It calculates the volume of the different tissues in each voxel. The final whole tissue volume in each image was obtained in litres.

2.6 Genetic data

2.6.1 *Genetic data collection*

DNA in the Family and Twin studies had been extracted from both blood samples and cheek swabs. During the baseline assessment of the AESOP study very few samples for genetic analysis had been collected. However, since patients were being re-contacted for a follow up evaluation, during this period I coordinated and collected additional DNA samples. For the AESOP study genetic samples were also collected from both blood samples and cheek swabs.

Blood samples were collected into an EDTA tube by a trained phlebotomist or medical professionals (including myself). The samples were stored in a -80^{o}C before DNA extraction. When the sample was collected with a cheek swab, subjects were asked to swab their inner cheeks with 10 sterile cotton swabs. These were stored at room temperature as advised by manufacturer until processed for DNA extraction.

2.6.2 *Genotyping*

2.6.2.1 *DNA extraction*

This step was carried out by colleagues at the Social Genetic Developmental Psychiatry centre (SGDP). The DNA was extracted with two different methods, depending on how the samples were collected. DNA was extracted from blood samples using a standard phenol chloroform extraction protocol. As for DNA extraction from cheek swab, this was done by an in house method (Freeman et al., 2003)

2.6.2.2 *DNA storage*

All samples were bar-coded, entered in the SADMAN database system and stored at -80^{o}C in freezer at the SGDP centre. Barcodes were created in sequence in order to identify each individual sample. Barcodes are made of letters and numbers and were matched to progressive numbers on original folders stored at the main building of the IoP in order to facilitate tracking. This numbers were allocated randomly to patients, relatives and controls to ensure blindness at the time of working in the laboratory and obtaining the results. Barcodes and subject identification numbers were matched to the original database and paper folders.

2.6.2.3 *Genotyping*

This was carried out by different methods. In my sample, the methods used were:

1-Internally with TaqMan at the SGDP centre

2-Externally by private companies
3-Externally with Genome Wide Association Study (GWAS)

In this section I will describe the TaqMan technique that I have performed. Additionally, I will highlight the other genotyping methods used for the different polymorphisms. The specific genotyping techniques used for each SNP are shown in table 2.4.

2.6.2.3.1 SGDP centre genotyping: TaqMan

In this section I will describe in more details the genotyping that I carried out for the *COMT* rs4680 polymorphism. *COMT* Val/Met (rs4680) was genotyped using TaqMan® SNP Genotyping Assays from Applied Biosystems, which is based on 5' nuclease activity and fluorescent nucleotide chemistry.

For the *COMT* genotyping, primers are at 900 nM final concentration.
PCR followed standard procedure as per Applied Biosystems TaqMan® SNP Genotyping Assays standard dry DNA protocol (Table 2.2; Figure 2.2).

Table 2.2: TaqMan® SNP Genotyping Assays standard dry DNA protocol

Reaction Component	Final Concentration	Volume (ul)
TaqMan Gene expression assay 20X Assay Mix	1X	0.1ul
DNA Template	10ng	2.ul
ddH2O		0.9ul
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)	1X	1ul

PCR reaction was carried out in the Applied Biosystems 7900HT Fast Real-Time PCR System machine. As per manufacturer protocol, the following conditions were applied:

STEP 1 - hold at 50C for 2 minutes

STEP 2 - denature at 92C for 15 seconds

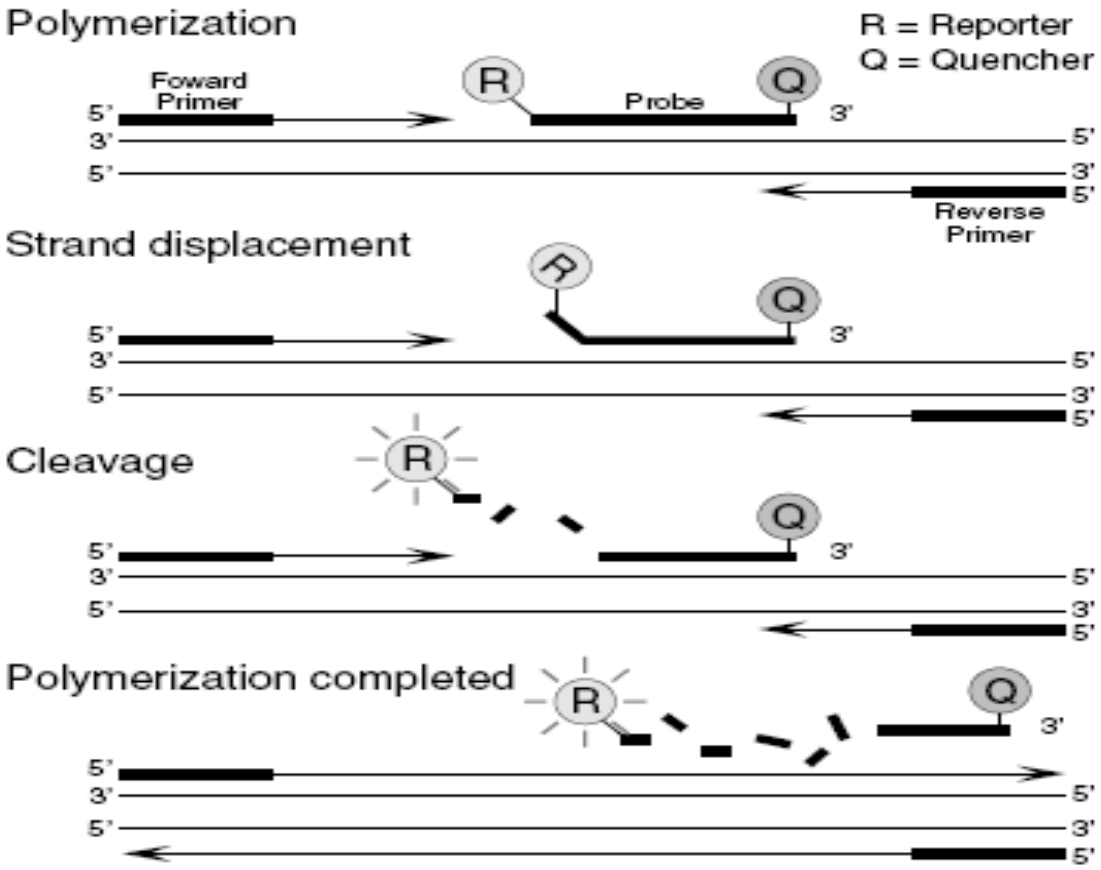
STEP 3 - anneal/extend at 60C for 1 minute

STEP 4 - repeat step 2 and 3 for 40 cycles.

Plates were then subjected to reading using the Applied Biosystems 7900HT Fast Real-Time PCR System.

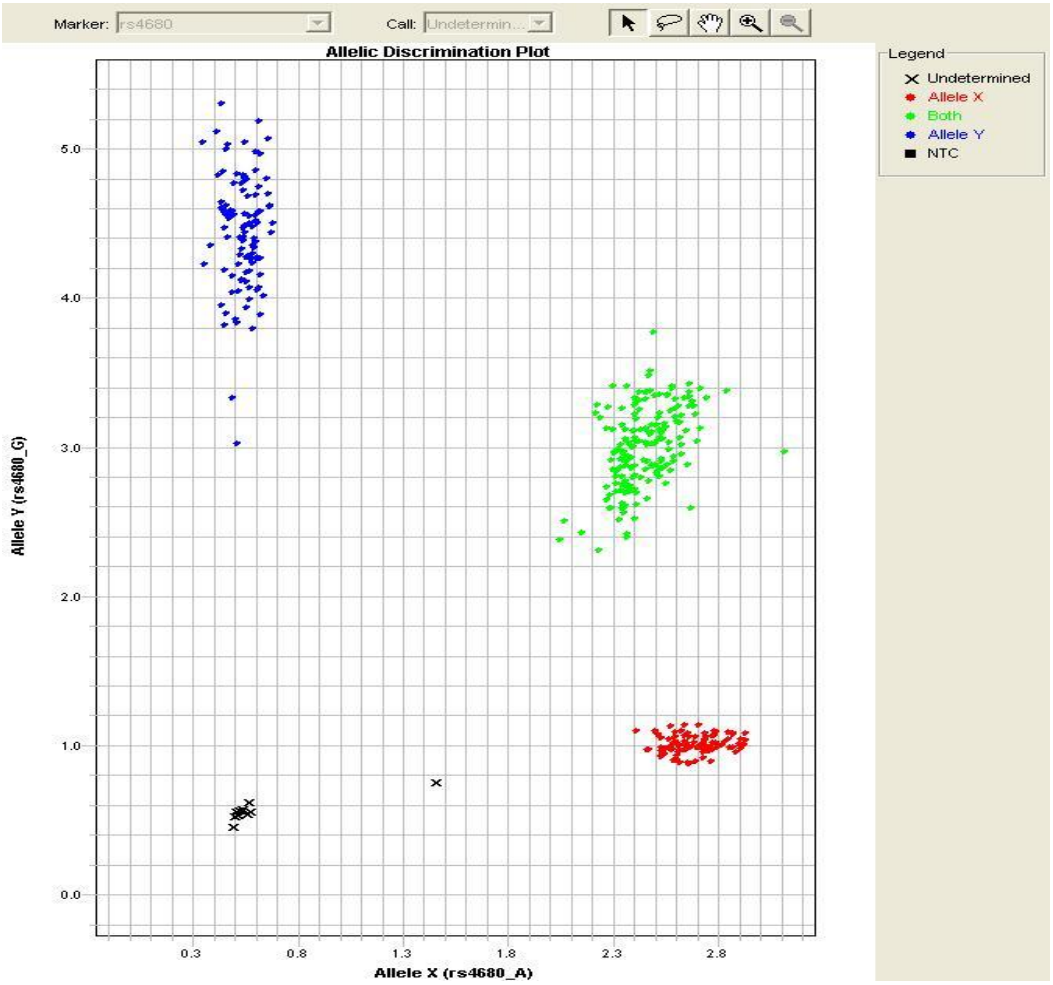
As shown in Figure 2.2, the TaqMan probe contains two types of dye: a reporter dye located at the 5' end and a quencher dye, located at the 3' end. The reporter dye is separated from the quencher dye during the reaction. The fluorescence increase can be detected only if the target sequence is present and has been amplified during the PCR reaction. Non specific amplification is not detected.

Figure 2.2 TaqMan Gene Expression assay Protocol shows the nuclease activity of the Polymerase system.



Presence of each allele is marked by colour and position on a generated plot according to the level of fluorescence present in each allele. Homozygotes are displayed in the expremities of the Y and X axes whereas, heterozygous are displayed in the middle. Allele discrimination can be visualized in a plot showing the two alleles and the genotype distribution groups (Figure 2.3).

`Figure 2.3 Allelic discrimination plot resulting from the TaqMan gene expression assay procedure. The two alleles are shown in the axis and in different colours: blue –homozygous G allele (Val/Val), red –homozygous A allele (Met/Met) and green –heterozygous A-G (Val/Met)



2.6.2.3.2 Genotyping conducted by external companies

NRG1: SNP8NRG221533 was genotyped using the primer extension SNuPe/genotyping platform Megabase (Amesham International, UK) (Williams et al, 2003)

NRG1: SNP8NRG241930 Taqman SNP assay was used for genotyping (kit format at http://www.appliedbiosystems.com)

NRG1-243177 (rs6994992) Taqman SNP assay was used for genotyping (kit format at http://www.appliedbiosystems.com)

*DTNBP1*P1757 (rs2005976) were genotyped using Kbioscience (https://kbioscience.co.uk) with a competitive allele-specific PCR system.

*DTNBP1*P1320 (rs760761) were genotyped using Kbioscience (https://kbioscience.co.uk) with a competitive allele-specific PCR system.

*BDNF*val/met polymorphism (rs6265) was genotyped using the primer extension SNuPe (Amesham International, UK), as previously described by Bramon et al (2006) in a previous study analysing part of this sample.

2.6.2.3.3 Genome Wide Association Study (GWAS)

Data were obtained from the GWAS carried out as part of the Wellcome Trust Case Control Consortium study of Psychosis Endophenotypes at the Institute of Psychiatry. Samples were genotyped using the Affymetrix 6.0 genotyping array by Affymetrix. Genotype calling and stringent quality control was performed by the Wellcome Trust Centre for Human Genetics, University of Oxford, UK. The SNPs selected for analysis in this thesis under the GWAS sample were selected according to the following approach:

1) I first carried out a literature review to search for specific genetic polymorphisms associated with brain volume, either in the general population or in patients with psychosis.

2) After the literature research, the approach to the GWAS data was based on candidate genes using two strategies:

A) SNPs that have been described to influence brain volume in psychosis:

*DTNBP1*_rs1047631
*DTNBP1*_rs875462
*NTRK2*_rs10868219
*RELN*_rs7341475;

B) SNPs that have been described to influence brain volume in healthy populations:

*MCPH1*_rs2305022
*MCPH1*_rs930557
*MCPH1*_rs1057090
*MCPH1*_rs2912016
*ASPM*_rs3762271
*OLIG2*_rs1059004

C) Finally, I included a number of SNPs recently described by Taal et al., (2012); Ikram et al., (2012) and Stain et al., (2012) in a GWAS study as implicated with brain volume in healthy individuals;

*SBNO1*_rs7980687
*HMGA2*_rs1042725
*CRHR1*_rs11655470.

3) SNPs and proxy

A number of the SNPs identified in the literature were not available from the GWAS data, so for these a proxy SNP was identified. Genetic association

tests depended on whether the sequence variant in question had a real functional effect on the phenotype, or works in probable linkage disequilibrium with a functional variant. These are identified by linkage disequilibrium, physical distance and/or membership in selected commercial genotyping arrays. Linkage disequilibrium simply represents the statistical correlation between two sequence variations due to a shared history. The extent of linkage disequilibrium is usually less than 200–300 kb. However, linkage represents a much more predictable force than disequilibrium, since it uses a well-known and precise mathematical form. To identify the proxies we used SNAP (http://www.broadinstitute.org/mpg/snap/). This site runs a pair-wise linkage disequilibrium which is pre-calculated based on phased genotype data from International HapMap project. A summary of these is presented in table 2.5.

Table 2.4 Genotyping methods

GENE	Genotyping method
SNP8NRG221533	primer extension SNuPe/genotyping platform Megabase
SNP8NRG241930	Taqman SNP assay was used to geneotype (kit format at http://www.appliedbiosystems.com)
NRG 243177	Taqman SNP assay was used to geneotype (kit format at http://www.appliedbiosystems.com)
DTNBP1P1757rs2005976	Kbiosciences With competitive allele specific PCR system

DTNBP1P1320 rs760761	Kbiosciences With competitive allele specific PCR system
BDNF rs6265	primer extension SNuPe Technology
COMT rs4680	Taqman and primer extension SNuPe Technology
DTNBP1_rs1047631	GWAS
DTNBP1_rs875462	GWAS
NTRK2_rs10868219	GWAS
RELN_rs7341475	GWAS
OLIG2_rs1059004	GWAS
MCPH1_rs2305022	GWAS
MCPH1_rs930557	GWAS
MCPH1_rs1057090	GWAS
MCPH1_rs2912016	GWAS
ASPM_rs3762271	GWAS
SBNO1_rs7980687	GWAS

HMGA2_rs1042725	GWAS
CRHR1_rs11655470	GWAS

Table 2.5 SNPs and their proxy and their r2 square

GENE	SNP	proxy	Distance bp* and r2
DTNBP1	rs875462	rs6937379	12439 bp 0.953
OLIG2	rs1059004	rs762178	1062 bp 0.901
MCPH1	rs930557	rs2440416	711 bp 0.891
MCPH1	rs1057090	rs2912057	4440 bp 0.875
MCPH1	rs2912016	rs2959798	5432 bp 0.844
ASPM	rs3762271	rs1360558	76469 bp 1.000
SBNO1	rs7980687	rs12322888	2848 bp 0.943

*base pair

2.7 Statistical Analysis

2.7.1 *Coding*

As the sample is composed by three different studies, I recoded all subjects to make the overall sample homogeneous and comparable. I coded all subjects with a unique number as belonging to a family. Additionally, each individual had a family number followed by a suffix that would indicate the uniqueness identity of the person to the family.

2.7.2 *Samples under investigation*

The whole sample was included in the analysis of brain volume, and three groups compared (patients, relatives, healthy volunteers).

For the case control and the genetic analyses, I selected the sample according to the hypothesis tested. The sub-samples for each analysis were selected as follows:

-In the case-control brain volumes comparison:

- Maudsley Family Study: I excluded all relatives
- Maudsley Twin study: In order to avoid familial confounders, I selected one subject of each twin pair. The selection of the twin individuals was done randomly. In order to maintain blindness while manipulating the data, I first identified and organized in an ascending order the twin pairs according to their unique identification numbers. Then, I selected the first subject of the pair from the organized list in the database as follows:

monozygotic concordant patient: one subject from each pair
monozygotic control twins: one subject from each pair.
monozygotic discordant twin: the ill subject of each pair.
dizygotic discordant pair: the ill subject of each pair
dizygotic control twins: one subject of the pair
The sample did not have any dizygotic concordant pairs

-In the genetic analyses

- From the whole sample, I excluded all non-Caucasian subjects
- Maudsley Twin Study sample, I selected 1 twin from each monozygotic pair. Dizygotic discordant twins were included and coded as a relative in the same family, sharing about 50% of the genetic loading.

Finally, in the sample I collected from the Maudsley Twin Study there were 6 twins of the pair that were excluded due to movement artect in the neuroimaging. Therefore, 6 twins were not paired.

2.7.3 *Brain volume analysis*

For these analyses I used STATA (version 10; Stata Corp, College Station, TX, USA), applying the regress command and combining robust and cluster options. In this, STATA uses the sandwich estimator of variance (Rogers 1993), which is model agnostic and robust against possible violations of the observations from assumptions of regression, including normality and independence of residuals. This approach has the advantage of correcting for type 1 error in cluster correlated data by correcting for observations between clusters (Binder, 1983). This method has been successfully used in previous studies for the assessment of potential endophenotypes familiarity (Bramon et al., 2004, 2005)

I ran two different analyses:

1- Cases vs Controls: For this analysis I excluded all the relatives from the Maudsley Family Study. From the Maudsley Twin Study, I excluded all the healthy twin of the pair. This was based on the rationale that the healthy twin of the pair is a relative of the ill twin. When the twins were concordant for the illness, I randomly selected one subject from the pair. I compared brain volumes between all patients as one group and the controls. The control group was the reference for the analysis in STATA. Subsequently, I compared the control group against patients with schizophrenia and then against patients with affective psychosis. Global grey and white tissue volumes differences between patients and controls were calculated using a linear regression analysis model with brain tissue volumes as the dependent variable, controlling for age, gender and Whole Brain Volume (WBV).

2- Whole sample comparison: I compared all patients with psychosis with the relatives and the control group. Additionally, I ran a familiality analysis of grey, white and whole brain volume in which the whole sample (patients, relatives and controls) was included. I carried out a linear regression analysis using standard errors that are robust against non-independence of observations from individuals within families (clusters) and against departures from normal assumptions. Patients and relatives were compared to the control group with their brain volumes as the dependent variable, controlling for age, gender and Whole Brain Volume (WBV).

2.7.4 *Statistical Genetic Analysis*

For the genetic statistical genetic analysis I used statistical software packages Stata version 10 (StataCorp LP, USA) and SPSS version 18 for Microsoft Windows (SPSS Inc., USA). The effect of candidate genes on brain volumes was examined using linear mixed models fitted with maximum likelihood methods. Correlations between members of the same family were accounted for by including random intercepts for families, which is needed to maintain correct type 1 error rates. The dependent variables were grey matter volume, white matter volume, and whole brain volume, while genotypes of the different SNPs were the main independent variables. In addition, all analyses were adjusted by the fixed effects of clinical group (patient, relative or control), age, sex and MRI protocol. Finally, for the genetic analysis, the power calculation was done using QUANTO: (http://hydra.usc.edu/gxe)

2.7.4.1 *Hardy Weinberg Equilibrium Test*

Hardy Weinberg Equilibrium (HWE) test was performed using chi-square test. Each SNPs was checked for deviation from HWE in the non-psychotic participants group. Due to the heterogenous nature of my sample that combined subjects from three different studies, HWE test was performed only in the Caucasian subjects to avoid ethnicity confounders (Kaufman et al.,

2001; Thomas et al., 2002; The International HapMap Consortium, 2003; Holliday et al., 2008).

2.8 Personal Contribution to the work of this Thesis

My main involvement has been with the AESOP study. I joined the AESOP study in 2005 and I have participated in many aspects of the follow up phase of the study, while also working on the cleaning up of the baseline data. Additionally, I have worked with the Maudsley Family Study and the Maudsley Twin Study by facilitating recruitment of subjects for the additional phases of the studies.

More specifically, I was involved in:

Recruitment

I coordinated, and personally contributed to, the collection and storage of DNA samples in the AESOP study. Since the AESOP study had only collected a small number of DNA samples in the baseline phase, I was responsible for tracing subjects, recruiting, consenting and extracting DNA samples during the follow up period. I personally collected about 40 to 50 DNA samples, and the rest of the samples were collected in collaboration with fellow researchers. This represented a significant increase in the number of samples.

Socio-demographic and Clinical Evaluation

In the AESOP study these assessments were already completed. However, to confirm the lifetime diagnosis and the progression of symptoms, some of the questionnaires were also carried out in the follow up stage. I administered the SCAN interview, Medication History, Medical History Checklist, Family interview for Genetic Studies (FIGS) to approximately 30 patients and 10 controls. I entered all the data for FIGS, and some data medication history, into the database.

MRI

MRI data collection had already been completed when I started this Thesis. However, the AESOP study was carrying out the follow up phase and I was involved in re-tracing and obtaining a second MRI scan of subjects for this phase. I single-handedly performed all quality checks, processing, and analysis of the MRI calibration study data and of the main MRI study presented in this Thesis.

DNA

In the AESOP study, I had responsibility for collecting DNA from participants and identifying samples acquired in the baseline recruitment phase. I personally collected approximately a fifty DNA samples. Finally, I contributed with the data-entry of the identification bar-codes for DNA samples. In the Maudsley Family Study and Maudsley Twin Study I collaborated with other researchers involved in these studies in subjects' tracing and recruitment. Once the subjects were recruited, I was responsible for obtaining consent for them to participate in the study and for obtaining DNA samples.

Chapter 3: MRI Calibration Study

3.1 Background

When looking at brain volumes from Magnetic Resonance Images scans there are generally 2 methodological approaches, manual and automated. The manual quantification of different brain volumes has been considered to be accurate and the *golden standard* for many years however; it has the disadvantages of being time consuming, it requires advanced knowledge of brain anatomy and may involve high inter-rater reliability when large samples are analyzed. In contrast, automated methods involve processing the images in an operator-independent way, with only minimal manual imputation. For this reason, is not only independent from inter or intra rater variability, but it has the additional advantage of analyzing larger neuroimaging data samples in shorter periods of time, hence providing increased statistical power in comparison to manual methods.

In fact, investigations of complex psychiatric disorders require large number of subjects to increase the statistical power. This can be achieved by combining MRI data obtained from multiple studies. However, this approach has also its own problems. For example, challenges of measuring and analyzing brain volume from images collected in different studies include:

1) they may differ slightly in their acquisition protocols;

2) if different scanners have been used, it is important to take into account between-scanner and within-scanner variability (Gradin et al, 2010);

3) the passage of time might change the quality of the images and requieres strict quality control (Simmons et al., 2011).

4) the application of manual methods of analysis may not be feasible, and therefore automated methods would need to be considered, although more sensitive to acquisition and inter-scanner variability.

In my thesis I analyzed a large sample of images obtained from 3 different studies at the Department of Psychological Medicine at the Institute of Psychiatry, King's College London. In order to analyze the images of these three studies together, I first performed a pilot reliability study to explore the comparability of MRI scans acquired across the different studies.

3.2 Aims of the pilot study

1) Obtain MRI brain images of the same 12 healthy volunteers using the acquisition parameters of the original MRI protocols of each study; in this way, I would estimate how much variations the same brain volume would display when different protocols were used;
2) Carry out automated segmentation of the images into grey, white matter and cerebrospinal fluid (CSF). These were in fact the main measures of interest in in my main study sample;
3) From the segmented brain images, obtain volume measures for total grey and white matter and whole brain volumes;
4) Assess the comparability across the 3 studies protocols.

3.3 Sample characteristics

Twelve healthy volunteers were recruited into this pilot study. These subjects underwent MRI scanning with the original protocols used in the 3 studies included in this thesis, more specifically: 1 protocol from the AESOP study, 1 protocol from the Family study, and 2 protocols from the Twin study (table 3.1). This preliminary work was called Aesop-Family-Twin (AFT) calibration project, and it allowed me to assess the variability between protocols of the 3 different studies from which I included subjects for the main thesis hypotheses. All healthy participants were screened for medical illnesses, neurological or psychiatric disorders, and met the standard MRI safety criteria. Informed consent was obtained from all participants. The participants were six women and six men, with a mean age of 29.8 years (SD $\pm$ 4.5). The demographic characteristics of the sample are described in table 3.1.

Table 3.1: AFT Calibration study sample demographic characteristics

Number	12
Gender (female/male)	6/6
Age (mean)	29.83 +/- 4.52

3.4 Magnetic Resonance Imaging

The MRI images were all obtained with a 1.5T General Electric Signa System scanner (Milwaukee, WI) at the Maudsley Hospital. For the volumetric analysis, a 3-D T1-weighted coronal spoiled gradient recalled echo scan (SPGR) was acquired according to each original study protocol.

For each subject 3 Magnetic Resonance Image (MRI) T1-weighted sequences were acquired, one for each original study protocol. The images were acquired between May 2006 and June 2007.

3.5 Acquisition protocols for the three studies

It is important to mention that during its duration, the Twin study had used 2 different protocols (parameters for both protocols are included in table 3.2). The first one was called “Long”, and the second protocol was called “Short”. This change of protocol was done to make the MRI data more compatible with data acquired from other studies carried out in the Department, and specifically with the Family study. To achieve this, the Twin study “Short” protocol used the same acquisition parameters used by the Family study protocol. For the purposes of this calibration analysis therefore only the “Long” protocol was included, since the “Short” protocol used the same parameters already included in the “Family study protocol.

The parameters of acquisition of each protocol are described in table 3.2. The protocols included in my pilot project were therefore the following:

- Protocol A (4_A): Ethnicity and Aetiology in Schizophrenia and Other Psychosis (AESOP)

- Protocol F (3_F): Maudsley Family Study (MFS)

- Protocols T (7_T: Maudsley Twin Study (MTS). This was called the "long" version of the MTS. I chose to first estimate the correlation of the Long Twin protocol because a larger number of subjects had been scanned with this sequence. However, the Maudsley Twin Study had also used the same protocol as the Maudsley Family Study (MFS) and this was called the "Short" Twin protocol.

Table 3.2: protocols acquisition variables

Protocol as original name	**FAMSCHIS**	**TWINSCHIS long**	**AESSOPC**
Name for calibration	3_F	7_T	4_A
Orientation	Coronal	Coronal	Coronal
TR	13.1	35	13.8
TE	5.8	5	2.8
TI	450	0	450
Flip angle	20	35	20
xdim	0.859375	0.78125	0.9375
ydim	0.859375	0.78125	0.9375
zdim	1.5	1.5	1.5

3.6 Image pre-processing

From the 12 health participants, 36 different images were obtained, 3 per subject. I performed quality control on all image data acquired, to increase consistency. This control ensured: full brain coverage, wrap around artifacts affecting the brain, motion artifacts, intensity inhomogeneity, adequate

grey/white matter contrast throughout the images, and any evident brain abnormality. All 36 images fulfilled criteria of good quality to be included in the calibration study.

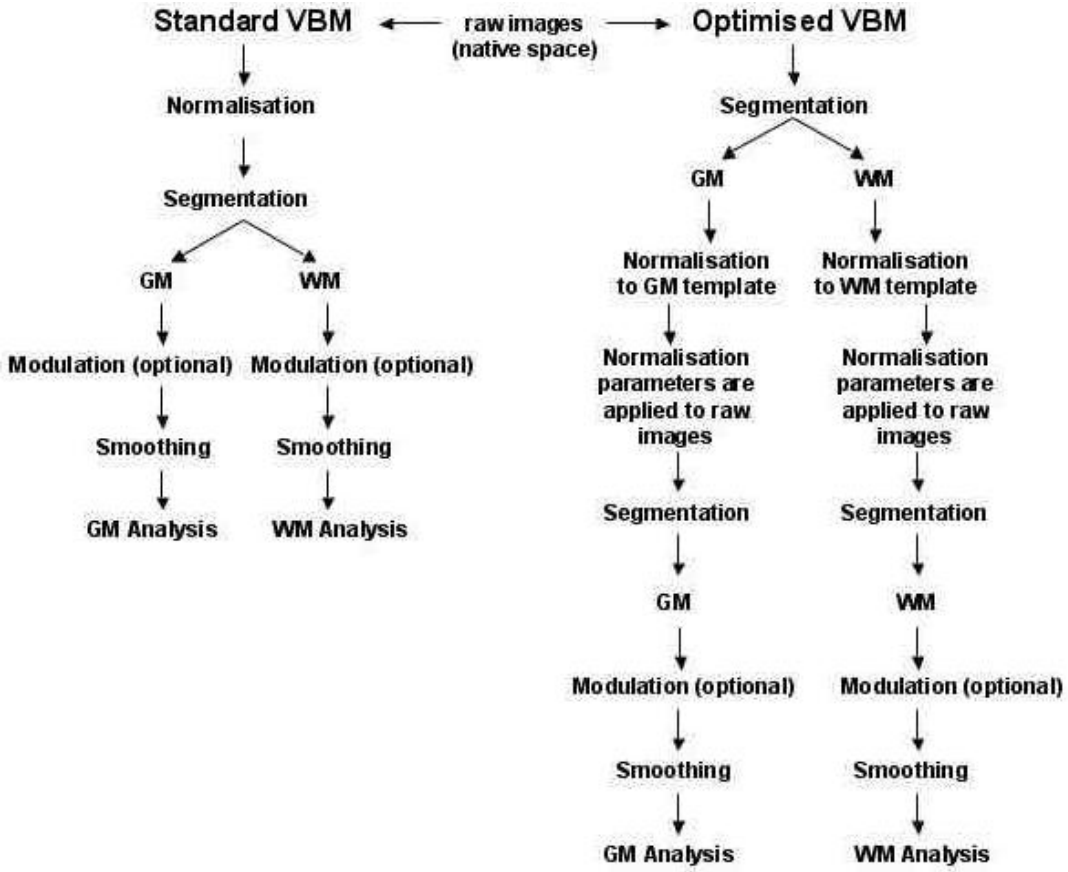
Images were processed using Sun workstation (Sun Microsystems, Mountain View, USA) using MAT-LAB (MathWorks, Natick, MA) and Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm/software/spm2). During the course of the calibration project the SPM method was updated and developed further. Therefore, for the first correlation analysis I used Statistical Parametric Mapping (SPM) version 5, while for the following 2 analyses I used the upgraded version, SPM8.

The full description of the image processing is described in Chapter 2. In summary (graph 3.1), SPM is a fully automated method used to examine structural MRI brain images with a voxel-based method (VBM) approach. VBM approach is used to identify differences in regional brain tissue, while taking into account large scale differences in anatomy and position. In the standard SPM method, this is achieved by spatially normalizing all the structural images to the same stereotactic space, segmenting the images into gray and white matter and finally smoothing the gray and white matter images for statistical analysis (Ashburner and Friston 2000).

The newest versions of SPM use the optimized VBM method (Good et al., 2001). This process includes one more steps than the standard method. These are summarized as: 1) Segmentation into grey and white matter tissues 2) Spatial Normalization and realignment to grey and white matter templates 4) Smoothing and 5) Segmentation into component tissues: grey and white matters, cerebro-spinal fluid (CSF) and dura/blood vessels.

The optimized method has the benefit of reducing tissue classification in the segmentation process. In addition, a further step in the pre-processing of

neuroimaging called “modulation” can be added to compensate the likely introduction of volumetric differences after warping a series of individual images to match a template during spatial normalization. The modulation process is particularly useful, when trying to identify regional differences in the volume of a specific tissue, either gray or white matter, as it requires the information about absolute volumes to be preserved. This step involves multiplying the spatially normalised grey or white matter by its relative volume before and after the normalization process. With this additional step, the volume measurements of gray or white matter structures can be more accurate (Mechelli et al., 2005).



Graph 3.1: Flow diagram of the preprocessing steps in standard (left) and optimized (right) VBM. GM = gray matter images; WM = white matter images from Mechelli A et al., 2005

3.7 Results

3.7.1 First analysis

3.7.1.1 Image Processing

All images for the AFT calibration project were acquired in coronal orientation. However, SPM requires image files to be in axial orientation. Therefore, all images were first converted to axial orientation. I carried out this step by running an ad hoc script prepared by the Centre for Neuroimaging Sciences.

Once the images had been converted into axial orientation, I was able to run and estimate the brain volumes. The pre-processing was done with SPM version 5.

The SPM5 segmentation was run using the default settings. The quality of the segmented images was visually checked by me. More specifically, I examined the quality of both the modulated and unmodulated segmented brains. All segmented images were of good quality to be included in the analysis. From the probability maps obtained, volumes of grey and white matters and Cerebro-Spinal Fluid (CSF) were calculated as both voxels and volumes. In addition, Whole Brain Volume (WBV) was calculated by adding the white and grey volumes. At the time of this analysis SPM was still undergoing optimization. Therefore, to check for consistency and reliability of the segmentation, and establish whether any of the two should be chosen against the other, I also compared volumes obtained with the modulated and unmodulated maps.

3.7.1.2 Correlation analysis 1

All statistical analyses were carried out using SPSS package version 13 (SPSS Inc., Chicago, IL).

I first estimated the correlation between the two measures of volume, voxel versus litres, for each subject for the 36 images obtained. This was to ensure they were expressing the same measure. The Pearson's r correlation was 1 across all data.

The second analysis evaluated the intra-class correlation among the 3 protocols, on both the modulated and unmodulated data. This was done to assess if it was worth adding an additional modulation step into the pre-processing pipeline. Since the intra-class correlation coefficient increases when the correlation between the items (in my case the volumes) also increases, a level above 0.9 is considered significant and is therefore desirable. I found that the correlation among protocols was higher, although the difference was not significant, for the modulated than for the unmodulated data. This was somehow not surprising, since the modulation corrects for the effects of spatial normalization. Results are shown in table 3.3. Therefore, for subsequent analyses, I used the modulated images.

Table 3.3 Results of brain volumes correlational analysis 1

	Grey matter	White matter	CSF
ICC modulated	.89	.99	.86
ICC unmodulated	.85	.96	.83

Once I decided to work with the modulated data, I explored the correlation in grey and white matter volumes between the AESOP, Family, and "Long" version of Twin protocols. I chose to first estimate the Long Twin protocol because a larger number of subjects had been scanned with this sequence (in comparison to the Short Twin sequence). Therefore, if this sequence proved to be compatible with the ones obtained from the other studies, I would be able to

include more subjects, and hence have a higher statistical power to investigate the hypotheses of the study.

3.7.1.3 Intra-class correlation analysis:

The preliminary results on grey and white matter volumes are described on table 3.4.

Table 3.4: Gray and white matter volumes on the three studies and Intra-class Correlation Coefficient (ICC)

Study	Grey volume (mean voxels)	*Standard Deviation*	*ICC*
AESOP	84358	6957.53	0.894
Family	84865.17	6545.87	(0.688 - 0.971)
Twin	87074.36	8635.08	

Study	White volume (mean voxels)	*Standard Deviation*	*ICC*
AESOP	55731.75	6957.53	0.977
Family	58316.32	6545.87	(0.934 - 0.994)
Twin	62131.86	8635.08	

I calculated the intra-class correlation coefficient across the 3 protocols using the modulated data. I evaluated this using Cronbach's alpha. This is a coefficient, between 0 and 1, that is used to rate the internal consistency (homogeneity) or the correlation of the items in a test.

This analysis showed that while white matter volumes were significantly correlated, with a Cronbach's alpha of 0.977, there was a worse correlation across grey matter volumes, for which the Cronbach's alpha was 0.894. I then looked at the correlation between each study with Paerson Correlation Coefficient. The results of these analysis showed that there was good correlation between the Maudsley Family Study protocol and the AESOP protocol (grey matter: r=0.84, p-value: 0.003; white matter: r=0.95, p-value: <0.001). However, the correlation was significantly worse for both grey and

white matter volumes between the Maudsley Twin Family Study (MTS) “Long” version protocol and the AESOP protocol (grey matter: r=0.23, p-value: non-significant; white matter: r=0.44, p-value: non-significant) and with the Maudsley Family Study (grey matter: r=0.03, p-value: non-significant; white matter: r=0.55, p-value: non-significant)

3.7.1.4 Conclusions of the first analysis

In summary, the first analysis of the correlation among protocols showed that:

1) Although with little effect, modulated data showed better segmented volumes correlation between protocols than unmodulated data, and therefore this pre-processing step should be incorporated in further analyses.

2) In line with previous studies, the CSF segmented volumes showed more heterogeneity across protocols than grey and white matter volumes (Good et al., 2001).

3) Given the unreliability of the CSF segmentation measures with this fully automated pre-processing method, CSF volume analysis was excluded from the computation of Total Intracranial Volume (TIV).

4) In order to assess if the reliability and comparability could be increased among different protocols by manual reorientation, I decided to also re-orient the images manually.

3.7.2 Second analysis

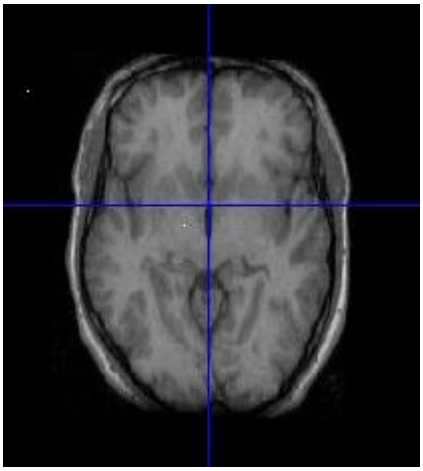
In this analysis I intended to explore further the poor correlation between the three protocols I identified in the previous section. In this phase of the calibration project I also corrected the MRIs for intensity in-homogeneities, and I used an updated version of the SPM8 software.

3.7.2.1 Image Processing

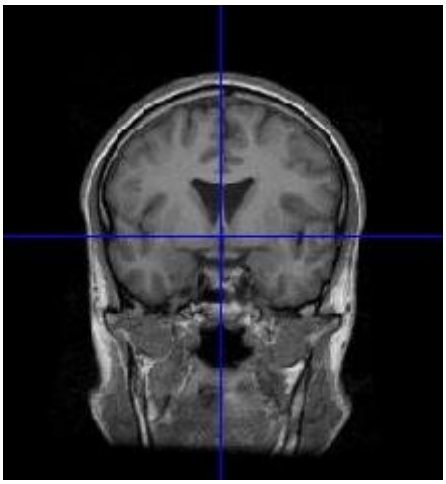
Magnetic resonance signal intensity form homogenous tissue is not always uniform. This is due to artifacts introduced by differences in the sensitivity of the scanner reception coil, non-uniform excitation and induced eddy currents (Sled et al 1998). It was particularly important to apply this correction as in my main study I was going to include MRI images obtained with different protocols and at different times. Therefore, to increase the accuracy of the segmentation and the subsequent brain volumes extraction, I applied the N3 algorithm (Sled et al., 1998) to improve the intensity non-uniformity of the images. Additionally, this method has been shown to improve VBM if used prior to the SPM analysis (Acosta-Cabronera et al. 2008). The N3 function algorithm was applied using a script that corrects intensity non-uniformities and also converts the images from coronal to axial orientation and also from UNC to Analyze format. Therefore, all the images obtained after this step were ready to be processed with SPM8.

I also addressed the previous conclusion that poor correlation in tissue segmentation across protocols could be related to the orientation parameters and alignment of the images. For these reasons, in this second analysis I manually re-oriented all the images, using the key landmarks of the Talairach and Tournoux atlas (1988). I manually reoriented each image along the anterior-commissure posterior-commissure axis in the sagittal plane and along the inter-hemisferic fissure in the coronal and axial planes. The guide-brain image I used to reorient my scans is shown in Graph 3.2; 3.3 and 3.4. The reorientation was done using the reorientation tool of SPM, where the image can be reoriented by manually manipulating the parameters. Once I was satisfied with the orientation I saved the image.

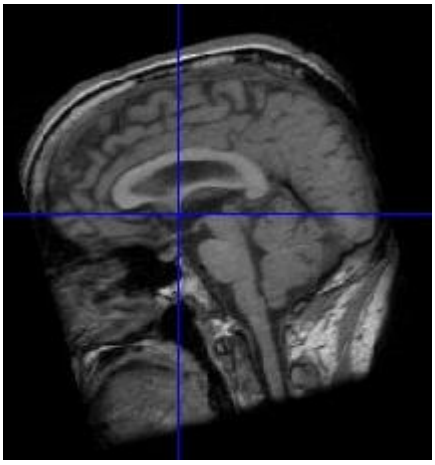
Graph 3.2 Axial orientation



Graph 3.3 Coronal orientation



Graph 3.4 Sagittal orientation



These images were then used for tissue classification and segmentation. As I applied the N3 algorithm to the images, for the SPM8 segmentation I selected, for the "bias regularization" field: "extremely heavy regularization" (10) and for the "Bias FWHM": "150mm cutoff" as these are the recommended parameters in the SPM manual. The remaining parameters were the SPM8 default ones. From the probability maps obtained, volumes of grey and white matter and Whole Barin Volume (WBV) were calculated as both voxels and volumes. WBV was calculated by summing the grey and white matter volumes. As mentioned in the previous section, in this analysis I did not include the CSF values as these had proved to have a high coefficient of variation (Good et al 2001).

As in the previous analysis, I visually checked the quality of the segmented images before the statistical analysis. All segmented images were of high quality and therefore included in the analysis.

3.7.2.2 Correlation analysis 2

All statistical analyses were carried out using SPSS package, version 15 (SPSS Inc., Chicago, IL). As in the previous analysis, although the intra-class correlation (ICC) was generally good and showed a slight improvement for grey matter volumes, no considerable changes were observed after applying the correction. This analysis showed that while white matter volumes were still significantly correlated, with a Cronbach's alpha of 0.977, there was a poor correlation across grey matter volumes, for which the Cronbach's alpha was 0.92. Even more, when looking at the between-protocols correlations with the Twin "Long" protocol, the results were still unsatisfactory. I then checked the correlation between studies, and found that there was good correlation between the Maudsley Family Study protocol and the AESOP protocol, Pearson correlation: grey matter: r=0.84, p-value: 0.003; white matter: r=0.95, p-value: 0.000. However, there was poor correlation in grey and white matter

volumes between the Maudsley Twin Study (MTS) long version protocol and the AESOP protocol grey matter: r=0.33, p-value: non-significant; white matter: r=0.44, p-value: non-significant and with the Maudsley Family Study grey matter: r=0.03, p-value: non-significant; white matter: r=0.55, p-value: non-significant

Given the lack of improvement in the correlation results despite more meticulous approach, particularly with the Maudsley Twin long-protocol, I considered and reviewed the acquisition parameters again (Table 3.5). The different parameters used in the Twin-Long protocol seemed to provide the most obvious explanation for these differences.

Table 3.5: Protocol acquisition parameters highs in red the protocol from Maudsely Twins Study as the one different from the other two protocols (AESOP and Maudsley Family Study)

Protocol as original name	**FAMSCHIS**	**TWINSCHIS long**	**AESSOPC**
Name for calibration	3_F	7_T	4_A
Orientation	Coronal	Coronal	Coronal
TR	13.1	35	13.8
TE	5.8	5	2.8
TI	450	0	450
Flip angle	20	35	20
xdim	0.859375	0.78125	0.9375
ydim	0.859375	0.78125	0.9375
zdim	1.5	1.5	1.5

3.7.2.3 Conclusions of second analysis

Since the aim of the AFT calibration project was to establish the comparability of MRIs obtained across 3 different studies, I explored the possibility of including the second, different, protocol used in the Twin study: the “Short” protocol. As mentioned above, during the recruitment period, The Maudsley Twin Study used 2 protocols: the “long version” which was included in the AFT calibration study, and the “short version”, which had the same acquisition parameters as the Maudsley Family Study. Including data collected with this short protocol meant that I would only have to explore the correlation of 2 protocols. As I decribed above, I originally considered the MRIs obtained with the long version because there was a larger number of subjects on whom MRI and genetic data relevant for my main study were available. I looked at the potential number of MRI images that could be included in my study if the short version of the Maudsley Twin Study was included. There were 100 MRIs obtained with the short version protocol (same acquisition parameters as the Maudsley Family Study). Therefore, I decided to consider including these in my main study.

In my next analysis, I will refer to the short version of the Maudsley Twin Study as the Maudsley Family-Twin protocol, as the protocol was the same as the one of the Maudsley Family study.

3.7.3 Third analysis

3.7.3.1 Image Processing

In order to increase the quality of the segmentation and correlation across protocols, in this phase I first applied the same processing steps to the images as described in the previous section. However, after applying the N3 algorithm, rather than reorienting all the images, I reoriented the images acquired with the Maudsley Family-Twin protocol and realigned the images acquired with the AESOP protocol to the reoriented Maudsley Family-Twin protocol images.

All the realigned images were segmented applying the same parameters as described in the previous analysis. The volumes were extracted by segmenting the brain images with SPM8.

3.7.3.2 Correlation analysis 3

I explored the correlation between volumes using SPSS. I run a between-protocol reliability test again to estimate the intra-class correlation coefficient (ICC) for the volumes of interest. Using the Maudsley Family-Twin Study and AESOP protocols, the correlation between studies improved significantly, as shown in Table 3.6. The Pearson's correlation between the AESOP and the Maudsley Twin-Family protocol was, for the grey matter r=0.98 (p=000), and for white matter r=0.95 (p=000). The reliability test was conducted and the Cronbach's Alpha was above 0.98 for all volumes.

Table 3.6 Results of correlation between studies after in the third analysis: Paired Samples Correlation

	N	Correlation	Sig
Pair 3_F&4_A Grey matter vol	12	.980	.000
Pair 3_F&4_A White matter vol	12	.995	.000
Pair 3_F&4_A Whole brain vol	12	.992	.000

3.7.3.3 Conclusions of the third analysis

Using MRIs acquired with more similar acquisition parameters increased the correlation of the brain volumes obtained with SPM. I therefore decided that, for my main study analysis, only the individuals who had been scanned in the Twin study using the Maudsley Family-Twin sequence were to be included.

3.8 Discussion

I described a method that can be applied to combine MRIs from different studies. Twelve healthy volunteers were scanned with the same scanner and acquisition protocols used in the original studies. All the images were checked for quality, explored for differences between modulated and unmodulated data, and processed to obtain grey, white and CFS volumes.

I carried out 3 different analyses looking at the correlation and reliability of brain volumes when combining data obtained from three protocols in the same scanner. In the firsts analysis, although the intra-class correlation among the 3 studies was good (above 0.8 for grey matter and 0.9 for white matter), the correlation between studies was below 0.5. This was particularly evident with the Maudsley Twin Study long-protocol in comparison with the other two protocols.

In view of this, I then modified the image pre-processing. I reoriented the images manually and also applied an additional intensity non-uniformity correction with the N3 function. Despite these additional steps the correlations continued to be unsatisfactory. After reviewing the acquisition paramenters, it appeared that the Maudsley Twin long-version protocol accounted for the poor correlation. I then decided to use the short version of the Twin protocol, obtaining better volume comparability.

3.9 Conclusion

This comparability study suggests that, when using SPM, modulated images provide more robust and reliable correlation of volumes obtained with different protocols. SPM is a fully automated method that adds a great value to the pre-processing and analysis of a large number of neuroimaging data. However, similarly to other automated methods, this also has some disadvantages. For instances, although SPM runs an intensity non-uniformity correction during the segmentation pre-processing, it has been shown that the application of

another step by applying N3 algorithm ensures better segmentation, and therefore should be applied to increase reliability (Acosta-Cabronera et al. 2008). This is particularly important when analyzing images that have been obtained over a long period of time, as the sensitivity of the scanner reception coil, induced eddy and non-uniform excitation can change in time (Sled at al., 1998). In addition to this, the orientation and rotation of images along the same axes is also important and should be checked and corrected if necessary.

Finally, I believe that the most important conclusion for this study is that when seeking to combine MRI images from different studies/centers, it is important to first explore comparability of MRI scanners and establish correlation between data acquired with different parameters. This can be more easily assessed by scanning the same subjects in the different scanners and with the different protocols. This will allow to take into consideration the variability that may accounts for subjects differences. In fact, as shown in this calibration study, even small differences in acquisition parameters can play an important role when analyzing multicentre or multi-study scans.

Chapter 4: Results: Demographic and clinical characteristics

4.1 Methods

I collected information from subjects recruited from three studies conducted in the Division of Psychological Medicine: Aetiology and Ethnicity of Schizophrenia and Other Psychosis (AESOP), Maudsley Family Study (MFS), and the Maudsley Twin Study (MTS) of psychosis. All subjects had a structural MRI brain scans and a DNA sample.

4.2 Sample

A total of 541 subjects were included from the three studies (Table 4.1).

4.2.1 *Aetiology and Ethnicity in Schizophrenia and Other Psychoses (AESOP)*

A total of 281 patients met the inclusion criteria and were invited to participate; 90 of these refused to take part in the investigation. Of the 191 patients who participated in the study, 90 disengaged before completing the assessments, and a total of 101 subjects underwent an MRI scan in the baseline recruitment process. A total of 110 healthy volunteers were recruited from the same sociodemographic area to match patients in terms of gender, ethnicity, and years of education and 96 agreed to have an MRI scan. From this final sample, I ensured that all images were of a good quality, and following this, I was able to include them all in my study.

4.2.2 *Maudsley Family Study*

In the original sample from the Maudsley Family Study, the age range was broad, and included subjects over the age of 68 years. Since in the other 2 studies included in my thesis the maximum age for inclusion was 65 years, to make the sample more homogeneous for statistical analysis removed from the analysis subjects older than 68 years of age. These were: 5 Relatives (ages: 80, 75, 73, 72 and 69 years old) and 1 Control (69 years old). After these

subjects were excluded, the final sample of my study reached a total number of 535.

4.2.3 *Maudsley Twin Study*

The final study groups consisted of 30 pairs of MZ twins concordant for schizophrenia or schizoaffective disorder; 21 pairs of MZ twins and 12 pairs of DZ twins discordant for schizophrenia, in which the co-twin was free of any psychotic illness. Finally, 55 pairs of MZ and 18 pairs of DZ control twins with no personal or family history of a psychotic or schizophrenia spectrum disorder up to the second-degree relatives were also included. From the images obtained in this study, I excluded 24 subjects that were identified as having acquisition parameters not compatible with the rest of the sample. In addition, 1 subject of a pair was excluded due to poor image quality. This left MRI scans from 40 pairs, 5 single affected twins and 1 single non-affected twin for inclusion in the analyses.

Table 4.1: whole sample distribution according to study.

	AESOP	Maudsley Family Study	Maudsley Twin Study	Total
Patients	101	82	42	225
Relatives	-	111	19	130
Controls	96	58	26	180

4.3 Statistical Analysis

All demographic statistical analyses were carried out using SPSS package version 13 (SPSS Inc., Chicago, IL). I used chi square (x^2) for non-parametric variables and t-test or Analysis of Covariance (ANCOVA) for the analyses of parametric variables.

4.4 Results of the whole sample

4.4.1 *Sample demographics*

For this analysis, the final sample was composed of a total of 225 cases, 130 relatives and 180 healthy volunteers. The patient groups was composed by 144 subjects with a diagnosis of schizophrenia, 69 subjects with an bipolar disorder and 12 subjects with other psychosis. The demographic characteristics of the final sample are outlined in the tables 4.2 and 4.3

Table 4.2: Clinical characteristics of the sample

	Schizophrenia	Affective Psychosis	Other psychosis	Relatives	Controls
AESOP	59	31	11	-	96
MFS	43	38	1	111	58
MTS	42	-	-	19	26
Total	144	69	12	130	180

4.4.1.1 Gender: There were significantly more males in the patients group (x^2=11.06; p=0.001). However, relatives and controls were well matched for gender (x^2=1.01; p=0.29 and x^2=1.3; p=0.25 respectively), with a larger number of male subjects in both groups. There was a significant gender difference within diagnoses in the patients group, with the bipolar disorder group having more females than males in their group (x^2=11.06; p=0.001).

4.4.1.2 Height: There were no significant differences in height among patients, relatives, and healthy controls.

4.4.1.3 Handedness: All groups had a majority of right handed subjects.

Table 4.3: Demographic characteristics of the final sample.

	Patients	Relatives	Controls
Subjects *n*	225	130	180
Gender *female/male, n*	87/138	63/67	80/100
Ethnicity *n* (Caucasian/black-african/black-caribbean/other)	162/17/27/19	129/0/0/1	128/14/25/13
Mean age, years *(SD)*	32.2 (10.5)	44.3 (14.8)	33.6 (11.7)
Age range, years	17-64	16-68	16-68
Mean age, years *(SD) female/male*	34.5 (11.1)/ 31.2 (10.3)	47.4 (14.1)/ 40.6 (15.0)	32.9 (12.4)/ 33.7 (11.7)
Handedness*, n right (%)*	177 (77.3)	105 (80.8)	100 (81.3)
Mean height, cm (SD)	173.1 (9.2)	169.6 (10.4)	171.4 (8.9)

4.4.1.4 Age: To test for age differences I used t-test and Univariate Analysis of Variance. There was a significant difference in age between groups. Patients were significantly younger than relatives (t-test: t=-8.9, p=.000; 95% CI=-14.7 to -9.4). Relatives were significantly older than healthy volunteers (t=7.36, p=.000; 95% CI=8.2 to 14.2). There were no differences in age between patients and controls.

When age was compared across studies (table 4.4), there was significant difference in mean age across studies. Patients in the AESOP study were significantly younger (mean age=27.0, SD=7.96) than patients in the Maudsley Family Study (mean age=37.9, SD=10.5; T-test: p=.001, 95% CI=-13.7 to -8.08) and the Maudsley Twin Study: (mean age=33.31, SD 10.66; T-test: p=.018, 95% CI=-9.56 to -2.96). Also, controls from the Maudsley Family Study were significantly older (mean age=39.7, SD=14.59) than in the AESOP study (mean age=28.3, SD=7.7; T-test: p=.000; 95% CI=-15.37 to -7.35) and in the Maudsley Twin Study (T-test: p=.000; 95% CI=0.73 to 13.1). There was

no statistically significant difference between controls in the AESOP study and the Maudsley Twin Study. Relatives from the Maudsley Family Study (mean age=46.6, SD=14.3) were significantly older than those in the Maudsley Twin Study (mean age=31.2; SD=10.8; T-test: p=0.005, 95% CI=8.6 to 22.2). All these differences remained significant when corrected for gender.

Table 4.4 Age according to study

	Patients			Relatives		Controls		
Age	AESOP	MFS	MTS	MFS	MTS	AESOP	MFS	MTS
Mean years *(SD)*	27.0 (7.96)	37.9 (10.5)	33.3 (10.7)	46.6 (14.3)	31.2 (10.8)	28.3 (7.7)	39.7 (14.6)	32.8 (8.8)

4.4.1.4.1 Age and diagnosis: The results are shown in table 4.5. The Patients with schizophrenia were significantly younger than patients with bipolar disorder and the relatives (p=.008; 95% CI=-8.1 to -1.2 and p=.000; 95% CI=-15.9 to -1.1 respectively).

There was also a significant age difference between bipolar patients and those with other psychosis (p=.024; 95% CI=1.1 to 15.8) and the relatives (p=.000; 95% CI=-12.1 to -5.1). Finally, the control and the other psychosis groups were also significantly younger than the relatives (p=.000; 95% CI=-13.4 to -8 and p=.000; 95% CI=-24.26 to -10). However, when corrected for multiple comparisons with Tukey's HSD (Honestly Significant Difference) test, only the difference between relatives and the all the other groups remained significant.

Table 4.5: Age according to diagnosis

	Patients			Relatives	Controls
	Schizophrenia	Bipolar disorder	Other-psychosis		
Subjects *n*	144	69	12	130	180
Gender *female/male n*	41/103	43/26	4/8	63/67	80/100
Mean age, years *(SD)*	31.03 (9.75)	35.67 (11.68)	27.17 (7.77)	44.31 (14.8)	33.59 (11.7)

4.4.1.4.2 Age, gender and diagnosis: Female subjects were consistently older in all the groups. However, the differences were not significant when corrected for multiple comparisons with Tukey's HSD (Honestly Significant Difference) test.

4.4.1.5 Ethnicity: The sample was formed of subjects from different ethnic backgrounds. The description is represented in table 4.3. The majority of the participants were Caucasian (n: 419, 78.3%), followed by Afro-Caribbean (n: 52, 9.7%) then other ethnic background that would include Asian, Pakistani and Indian origins (n: 33, 6.1%) and finally, Black African (n: 31, 5.8%). Subjects from the Maudsley Twin Study were all Caucasian. There were 2 Asian subjects in the Maudsley Family Study. The rest of the variations in ethnicity were related to the AESOP study sample. The x^2 test showed a significant difference in ethnicity in the three groups where the Caucasian were significantly outweighed: patients group $x^2=263.8$ p: .000; relatives group $x^2=126$ p:.000 and healthy volunteers group $x^2=295.5$ p: .000.)

4.4.1.6 Medications:

I obtained information on medication at the time of the MRI on 134 patients. In the schizophrenia groups: 13 patients (8.9%) were on typical antipsychotics,

45 (30.8%) were on atypical antipsychotics, 1 (0.7%) on mood stabilizer, 3 (2.1%) on both mood stabilizer and antipsychotic, 12 (8.2%) on both antidepressant and antipsychotic and 1 on both mood stabilizer and antidepressant. In the affective psychosis group: 1 subject (1.4%) was on atypical antipsychotic, 14 (20.3%) on mood stabilizer, 10 (14.5%) on both mood stabilizer and antipsychotic, and 9 (13%) on both mood stabilizer and antidepressant. Finally, 23 patients (32.8%) were taking lithium.

4.4.1.7 Brain volume analysis

The brain volumes were normally distributed in the 3 groups in the whole sample. The results of brain volume according to group are described in each chapter accordingly.

Chapter 5: Neuroimaging results: Case-Control

5.1 Introduction

The literature on brain volumetric changes in patients with schizophrenia and bipolar disorder was described in Chapter 1: Introduction. In summary, there have been a number of studies that have also been the subject of various meta-analyses that consistently identify a reduction of whole brain and grey matter volumes in these patients. In contrast, the findings on white matter have remained inconsistent.

In this chapter I will describe the analysis of brain volumes that I have conducted comparing patients with psychosis and healthy volunteers.

I predicted that subjects with a psychotic illness would present with smaller whole brain, grey and white matter volumes than healthy volunteers. In addition, I predicted that the differences in brain volumes between patients with schizophrenia and healthy volunteers would be larger for whole brain and grey matter volumes than between patients with bipolar disorder and healthy volunteers.

5.2 Sample

In this chapter I will look at differences in total grey and white matter volume and whole brain volume in patients versus the control group. Therefore, to avoid the potential effect of familiality, for this analysis I selected cases and controls from the three studies excluding all the relatives included in the Maudsley Family Study. Additionally, from the Maudsley Twin Study I excluded 1 subject from each pair of monozygotic concordant twins in both the patient (11 subjects) and the control (10 subjects) groups. Finally, I excluded 1 subject from each pair of the dizygotic twin from the control group (3 subjects). The selection of the individuals was done randomly. I first identified and organized the twin pairs in ascending order according to their

unique identification number. Then, I selected the first subject of each pair from the organized list. Finally, as for the monozygotic discordant twins, I excluded all the healthy subjects of the pairs (12 subjects). The Maudsley Family Study and AESOP study inclusion criteria for the healthy volunteers was that they should have not had any family or personal history of psychotic disorder. Therefore, I included all the healthy volunteers from these studies.

The final sample used for the case-control analyses was composed by a total of 219 cases and 167 controls. The patient group included 138 subjects with a diagnosis of schizophrenia, 69 subjects with bipolar disorder and 12 subjects with other psychoses. This distribution is represented in table 5.1. The clinical and demographic characteristics of the sample are described in chapter 2.

Table 5.1. Case control sample

Group	Number of participants
healthy volunteers	167
schizophrenia	138
bipolar disorder	69
other psychoses	12

5.3 Magnetic Resonance Images analysis

The acquisition, pre-processing and analysis of the structural MRI images have been described in chapter 2.

Here, I first compared differences in brain volumes between patients group as a whole and healthy volunteers, by using linear regression. For the second part of this analysis, I divided the patients into two main diagnostic groups: schizophrenia (which included diagnoses of paranoid schizophrenia, schizophrenia NOS and schizoaffective psychosis), and bipolar disorder (which included a diagnosis of bipolar 1 and 2 disorder). Each diagnostic

group was compared to the healthy volunteers in a single analysis for each tissue measure. Each tissue volume was regarded as the dependent variable, while group, gender, protocol, age, height and whole brain volume were entered as covariates. To investigate the potential influence of years of illness on brain volume, I ran a partial correlation analysis between brain volumes and years of illness, correcting for age, gender and whole brain volume. All tests were two-tailed. The analyses were performed using STATA version 10 (Stata Corp., College Station, Tex).

5.4 Results

The unadjusted brain volume among the different clinical groups is described in table 5.2.

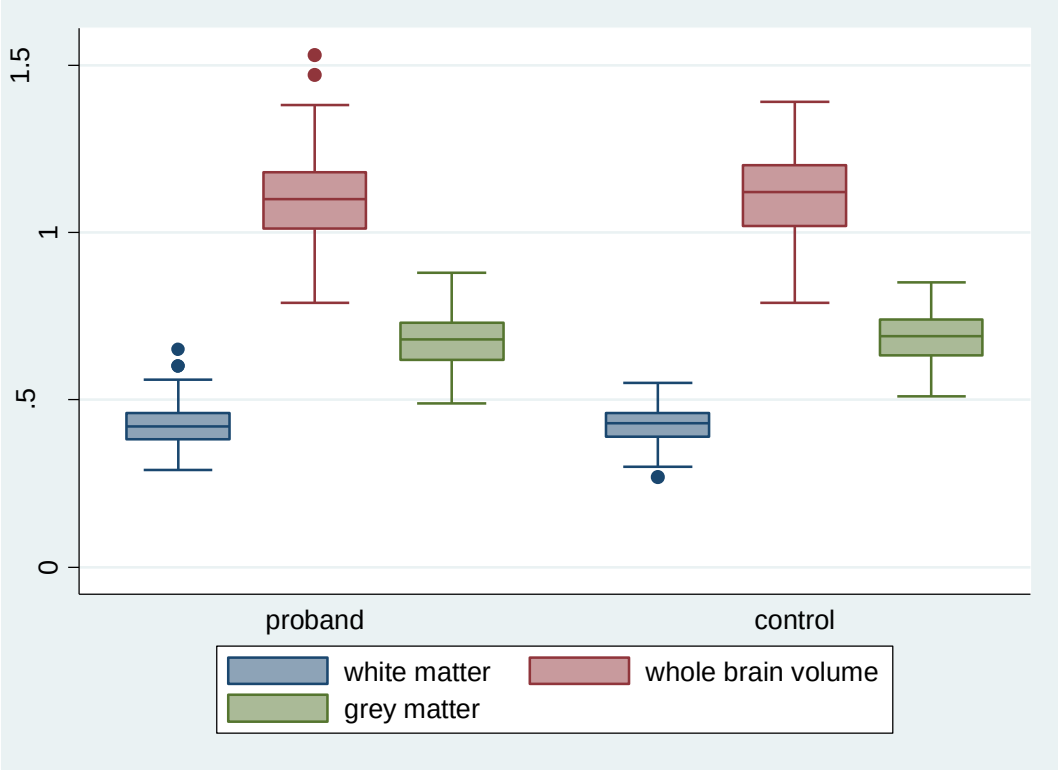
Table 5.2 Unadjusted brain volumes according to group

	Grey matter, mean litres (SD)	White matter, mean litres (SD)	TIV, mean litres (SD)
All patients together	.68 (.078)	.42 (.058)	1.10 (.13)
Schizophrenia	.66 (.076)	.43 (.058)	1.06 (.12)
Bipolar disorder	.69 (.075)	.40 (.050)	1.10 (.13)
Controls	.68 (.082)	.42 (.053)	1.10 (.13)

5.4.1 Brain volumes in all patients and healthy controls

There were no significant differences between patients and healthy volunteers in grey, white, or whole brain matter volumes. The box-plot in Graph 5.1 summarizes the results.

Graph 5.1: Brain volumes according to group



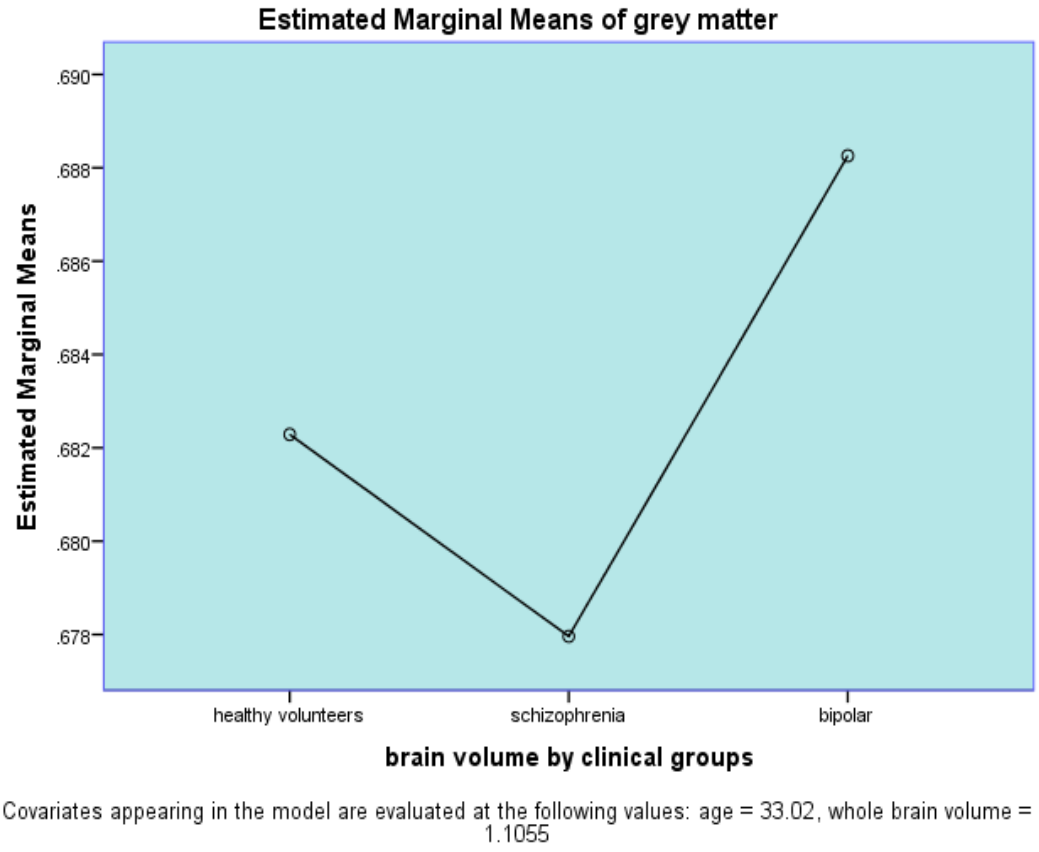
5.4.2 Brain volumes across different diagnostic groups and healthy controls

I explored whether there was any difference in brain volumes between patients with different diagnoses, and in comparison to healthy volunteers. For this analysis, I only included the two major diagnositic groups in the sample in order to reduce heterogeneity due to diagnostic classification. Therefore, I analyzed patients with schizophrenia or bipolar disorder and healthy volunteer groups.

5.4.2.1 Grey matter

Patients with schizophrenia showed significantly smaller grey matter volumes when compared to healthy volunteers (B=-.007, 95% CI=-.014 to -.0007; p=.03). Patients with schizophrenia also showed significantly smaller grey matter volume when compared to subjects with bipolar disorder (B=-.013, 95% CI=-.004 to -.022; p=.004). There was no significant difference in grey matter volume between patients with bipolar disorder and healthy volunteers.

Graph 5.2: Total grey matter volume according to clinical groups

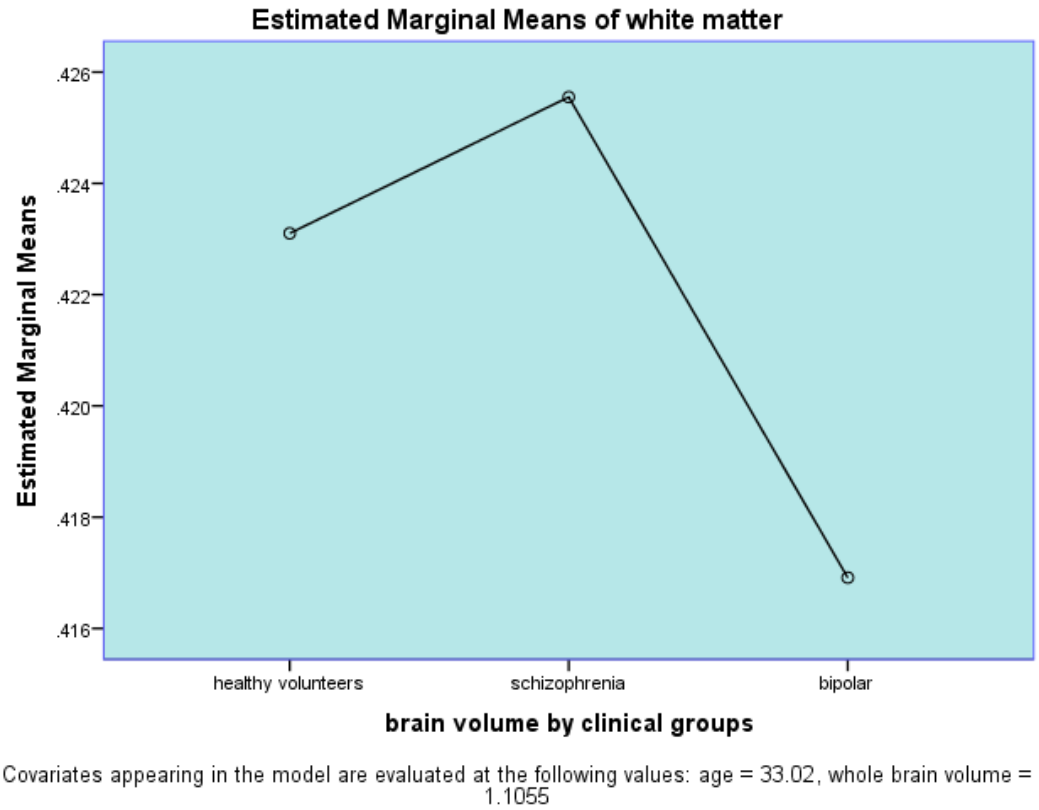


Covariates appearing in the model are evaluated at the following values: age = 33.02, whole brain volume = 1.1055

5.4.2.2 White matter

Both patients with schizophrenia and patients with bipolar disorder had significant differences in white matter volume when compared to healthy controls. However, these changes were in different directions. While patients with schizophrenia showed larger white matter volume than healthy volunteers, patients with bipolar disorder had a significantly smaller volume than healthy volunteers (B=.005, 95% CI=.0002 to .010; p=.04 and B=-.006, 95% CI=-.011 to -.0001; p=.04 respectively). When comparing patients with schizophrenia with patients with bipolar disorder, the difference was even more statistically significant. Patients with bipolar disorder showed smaller total white matter volumes than patients with schizophrenia (B=-.011, 95% CI=-.017 to .004; p=.001)

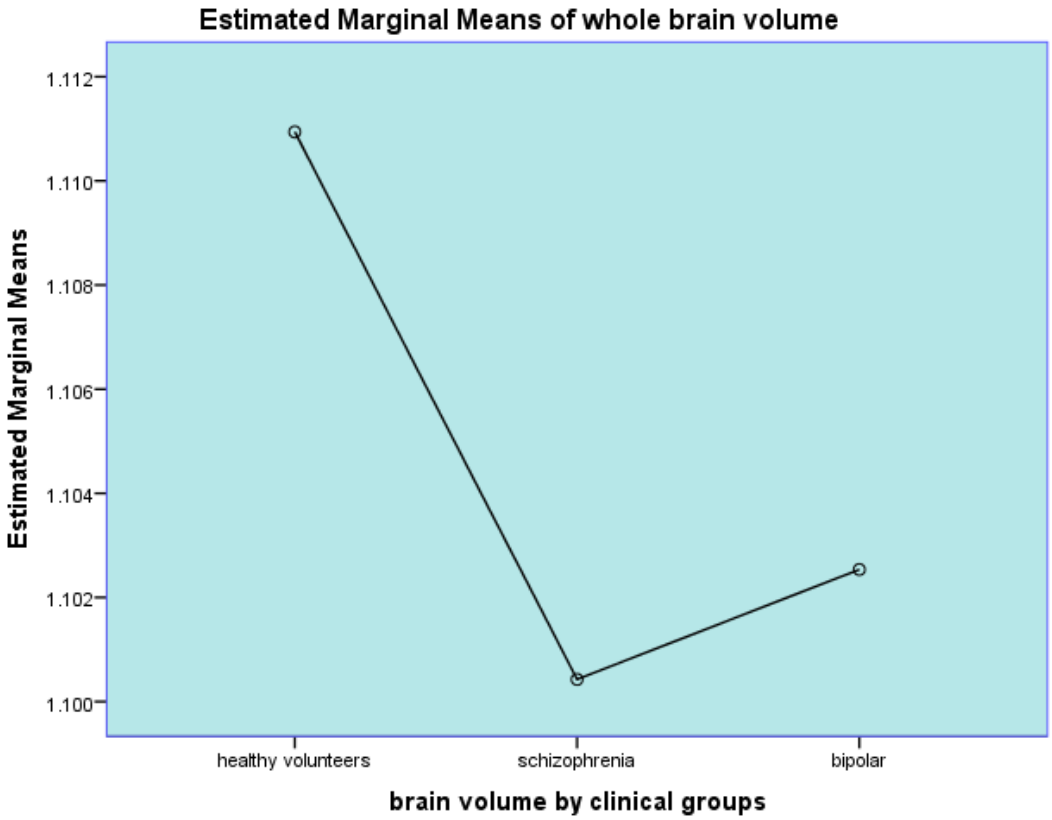
Graph 5.3: Total white matter volume according to groups



5.4.2.3 Whole brain matter volume

When comparing whole brain volume between different diagnostic groups and with healthy volunteers, only patients with schizophrenia showed a significant difference. They presented a smaller whole brain volume (B=-.024, 95% CI=-.048 to -.0001; p=.04) than healthy volunteers. It is worth noting that this difference seems to be related to the grey matter decrements since, as I previously described, white matter volume in this group was larger than the healthy volunteers. There were no differences in whole brain volumes between patients with schizophrenia and patients with bipolar disorder.

Graph 5.4: Whole brain matter volume according to groups



Covariates appearing in the model are evaluated at the following values: age = 33.02, gender = .58

5.4.3 Years of illness and brain volumes

There was no significant correlation between duration of illness (in years) and grey and white matter volumes. However, whole brain volume was negatively correlated with duration of illness (r=.229; p=.001).

When I explored if there were differences in the correlation between brain volumes and years of illness across the two main diagnostic groups, I found that, albeit at trend level (r=.231; p=.008), only schizophrenia showed a positive correlation between whole brain volume and the duration of illness.

5.4.4 Medication and brain volumes

I run a post-hoc partial correlation analysis looking at medication influence on brain volumes and this was not significant. However, this study was not designed to specifically answer that question. Hence, some of the brain matter changes could still be explained by the effects of different pharmacological treatments on the brain.

Chapter 6: Neuroimaging results: Brain volumes comparison with relatives and familiality analysis

6.1 Introduction

The inheritance of whole brain volume in schizophrenia has been described as between 66% and 97% (Peper et al., 2007; Rijsdijk et al., 2005). One of the most consistent findings in relatives of patients with schizophrenia has been the reduction in hippocampus volume (Kashavan et al 1997, Lawrie et al 1999; Johnstone et al., 2002) and whole brain and grey matter volumes (Boss et al., 2007). However, results remain inconsistent.

Twin studies have shown that co-twins with schizophrenia have smaller whole brain volumes than their healthy co-twin, who however also showed smaller brain volume than healthy control twins (Baare et al., 2001). This trend has been later replicated by another twin study by van Haren and colleagues (2004), which concluded that similarities in whole brain volume reductions were higher when pair members were more closely genetically related. A meta-analysis carried out by Boos and colleagues (2007) supports the initial findings of hippocampus and whole grey matter reductions in the relatives of patients with schizophrenia in comparison to healthy controls. In contrast, studies of relatives of patients with bipolar disorder have reported no morphological differences in comparison to healthy controls (McDonald el al., 2006). A study by McDonald et al., (2004) on structural brain endophenotypes, measuring genetic liability in families of psychosis, evaluated the genetic risks for brain volumes. The authors found illness-specific (i.e. schizophrenia or bipolar disorder) association in gray matter regional volume reductions, while white matter volume reductions were not illness-specific. These findings suggest that a genetic risk for psychosis is strongly related to brain structural alterations.

In order to understand brain deviations in complex psychiatric disorders like psychosis, studies have attempted to reach relatively large number of subjects to achieve the statistical power needed to detect subtle brain changes. In particular, some studies have explored the possibility of combining MRIs acquired with different scanners (Van Haren et al 2003; Meda et al 2008), and others have used a multicentre scanner design in patients with schizophrenia (Meda et al 2008; Segall et al 2009).

For my project I run a calibration study looking at the comparability of two different protocols and I addressed the difficulties of this approach in chapter 2. In the present chapter, I present the results of the analysis that uses computational morphometry to identify brain volumes differences in subjects affected with psychosis, first degree of healthy relatives and healthy volunteers. In addition, I explored the familiality of any brain deviation.

To my knowledge this is the first study that has compared patients with psychosis with a group of first degree relatives of patients with psychosis and healthy volunteers as a part of a multi-study design.

I predicted that subjects with a psychotic illness would present smaller brain matter volumes when compared with the relatives and healthy controls. Additionally, I predicted that the unaffected relatives would present brain reductions similar to those of patients but less prominent. A

6.2 Methods and Sample

In this section, I compare brain volumes among patients with psychosis, their unaffected first degree relatives and healthy volunteers.

The final sample used for this neuroimaging analysis included a total of 535 subjects: 225 patients, 130 relatives and 180 healthy volunteers. The full

description of the demographic of the sample has been described in Chapter 4: Clinical and Demographics Results. For this analysis I considered the relatives of each major disorder separately. The sample included 69 relatives of schizophrenia patients and 61 relatives of bipolar disorder patients. The summary of the sample demographics is shown in table 6.1.

Table 6.1: Sample summary.

Groups	N
Schizophrenia	144
Bipolar Disorder	69
Relatives of schizophrenia	69
Relatives of bipolar disorder	61
Controls	180

6.3 Magnetic Resonance Images

The acquisition, pre-processing and analysis of the structural MRI data were described in detail in chapter 2.

In summary, I combined neuroimaging obtained with two different protocols, and carried out the quality control and pre-processing of all the images.

6.4 Statistical Analysis

For this analysis I clustered the subjects into families. Therefore, each family was given an identification number and each subject a status identification code depending on whether they were patients, relatives or healthy volunteers. For these analyses I used STATA (version 10; Stata Corp, College Station, TX, USA), applying the regress command and combining robust and cluster options.

I first compared brain volumes between patients and relatives versus the control group, using linear regression analysis, with brain volumes as the dependent variable, and controlling for age, whole brain volume, protocol and gender. When analyzing whole brain volume, the results were corrected for height rather than whole brain volume.

Then, to explore whether familiality had an effect on brain volumes, brain volume values were subject to a mixed model analysis with fixed effect (group, age, gender, protocol and whole brain volume) and random effects (family). The linear mixed models provide an estimate of the between-family variability and the within-family variability. Therefore, I used the intra-family correlation coefficient (which measures the percentage of the total residual variability due to family differences) as an index of familiality. A likelihood ratio test was then used to assess the significance of the random (i.e., family) effect. The commands for these analyses were xtreg and xttest0. This method has been previously described (Bramon et al., 2004; 2005).

6.5 Results: Brain volume comparisons

The main brain volumes for each group are described in Table 6.2 and 6.3

Table 6.2 Unadjusted brain volumes according to group

	Grey matter, mean litres (SD)	White matter, mean litres (SD)	TIV, mean litres (SD)
Patients	.681 (.078)	.422 (.058)	1.10 (.127)
Relatives	.684 (.080)	.422 (.065)	1.10 (.136)
Controls	.684 (.082)	.423 (.053)	1.10 (.13)

Table 6.3 Uncorrected brain volumes according to diagnosis

	Grey matter, mean litters (SD)	White matter, mean litters (SD)	Whole Brain Volume, mean litters (SD)
Schizophrenia	.67 (.074)	.43 (.058)	1.08 (.12)
Relatives of schizophrenia	.67 (.078)	42 (.066)	1.10 (.14)
Bipolar disorder	.69 (.075)	.40 (.050)	1.10 (.12)
Relatives of bipolar disorder	.69 (.077)	42 (.056)	1.12 (.12)

6.5.1 Patients with schizophrenia, their relatives and healthy volunteers

When I analyzed grey and white matter and whole brain volumes, I found no statistically significant differences between patients with schizophrenia and their relatives or between relatives of patients with schizophrenia and healthy volunteers.

6.5.2 Patients with bipolar disorder, their relatives and healthy volunteers

When I compared grey and white matter volumes in patients with bipolar disorder, with those of their relatives, I found no statistically significant difference between the groups. However, patients with bipolar disorder had significantly smaller whole brain volume than their relatives (B=-.04; p= .01, 95% CI=-.01 to -.07). The comparison between the relatives of patients with bipolar disorder and the healthy volunteers did not reveal any signifficant difference in any of the three brain volumes analized.

All the analyses were carried out including the whole twin sample. In order to avoid duplication of genetically identical brains, I re-ran the analysis excluding 1 pair of the monozygotic twins following the same criteria as for the case-control analysis. This approach did not produce any change in the results.

6.6 Assessment of familiality

6.6.1 Schizophrenia Families

To estimate the familiarity of the different brain volumes, I compared the within-family variance against the between-family variance. In families with schizophrenia, for grey matter volume, the family clusters accounted for 48% of the total variance. For the white matter volume, the family clusters accounted for 27% of the total variance and for whole brain volume for 35% of the total variance. The results are summarized in tables 6.4, 6.5 and 6.6.

Table 6.4 Comparison of grey matter volume within and across schizophrenia families

	Standard Deviation	Variance
Between family factor	0.21	0.0004
Within family factor	0.20	0.043
Fraction of the total variance endorsed to the family group= **0.48**		P=0.000

Table 6.5 Comparison of white matter volume within and across schizophrenia families

	Standard Deviation	Variance
Between family factor	0.01	0.012
Within family factor	0.02	0.0003
Fraction of the total variance endorsed to the family group= **0.27**		P=0.004

Table 6.6 Comparison of whole brain matter volume within and across schizophrenia families

	Standard Deviation	Variance
Between family factor	0.06	0.004
Within family factor	0.09	0.007
Fraction of the total variance endorsed to the family group= **0.35**		P=0.000

6.6.2 Bipolar Disorder Families

In families with bipolar disorder, for grey matter volume, the family clusters accounted for 48% of the total variance. For white matter volume, the family clusters accounted for 43% of the variance and for whole brain volume for 22% of the total variance. The results are summarized in tables 6.7, 6.8 and 6.9.

Table 6.7 Comparison of grey matter volume within and across bipolar disorder families

	Standard Deviation	Variance
Between family factor	0.01	0.0001
Within family factor	0.014	0.0002
Fraction of the total variance endorsed to the family group= **0.48**		P=0.000

Table 6.8 Comparison of white matter volume within and across bipolar disorder families

	Standard Deviation	Variance
Between family factor	0.01	0.00016
Within family factor	0.01	0.0002
Fraction of the total variance endorsed to the family group= **0.43**		P=0.000

Table 6.9 Comparison of whole brain matter volume within and across bipolar disorder families

	Standard Deviation	Variance
Between family factor	0.042	0.0018
Within family factor	0.078	0.0061
Fraction of the total variance endorsed to the family group= **0.22**		P=0.015

As shown in the tables, the three volume measured are highly familial. However, out of the three, white matter, although statistically significant, seems to have less familial influence.

Chapter 7: Genes and brain volumes

7.1 Introduction

Currently, it is widely accepted that psychosis aetiology is based on a combination of genetic and environmental factors (Sullivan et al., 2003; Braff et al. 2007a). One recent approach to elucidate the pathways into these disorders has been the exploration of endophenotypes. As noted in chapter 1, endophenotypes are defined as a heritable disease trait (Gottesman & Gould, 2003), including a mong these brain morphometric deviations (Braff et al. 2007b). A number of global and regional volumes such as those of whole brain, grey and white matter, have been described as highly heritable, with an heritability estimated at around 85% (Sullivan et al., 2003; Peper et al., 2007; Kaymaz and van Os, 2009). These findings suggest that there is likely to be a considerable genetic influence in the brain differences observed in patients with psychosis. In addition, the progressive whole brain volume changes often reported in schizophrenia (Hulshoff et al., 2008) also are claimed to be related to genetic factors (Brans et al., 2008).

In this chapter I will explore the genetic influence of a number of relevant candidate genes involved in neurodevelopment of whole grey and white matter volume as well as in whole brain matter volume. I also explore the genetic influence of a number of candidate and neurodevelopmental genes that have been described in gene wide association studies (GWAS) to influence brain volume in healthy populations, namely SBNO1, NTRK2, HMGA2 and CRHR1. To my knowledge, this is the first time that the role of these genes has been assessed in a sample of patients with psychosis and their relatives.

7.2 Sample

The sample characteristics were fully described in Chapter 4: Results: Socio-demographics). For the genetic analyses, I included all subjects with

neuroimaging and genotyping data. In order to minimize the confounding effect of ethnic admixture, I only included Caucasian subjects from the AESOP, Maudsley Twin Study and Maudsley Family Study. Moreover, I selected twins with the same criteria I used for the neuroimaging analysis. The selection of the twins was described in detailed in Chapter 2: Aims and Methods. In summary, given that monozygotic twins share 100% of their genes, I randomly selected 1 twin of each monozygotic pair. Dizygotic twins were included and coded as part of the same family, since they share approximately 50% of the genetic loading, similarly to non-twin siblings. The final sample was therefore composed of 387 Caucasian subjects with genetic data. The sample was composed of 152 patients, 117 relatives and 118 healthy controls. The patient group included 99 patients with schizophrenia disorder, 48 patients with bipolar disorder and 5 subjects with another psychosis (psychosis NOS). I have summarized the demographics of the sample in Table 7.1.

Table 7.1: Sample demographics for genetic analysis

	Patient	**Relative**	**Control**
Total number = 389	152	117	118
male, n (%)	92 (60%)	51 (43%)	65 (55%)
Mean age (SD)	34.36 (10.8)	45.8 (14.5)	34.8 (17.7)
Diagnosis			
Schizophrenia	99	NA	NA
Affective Psychosis	48	NA	NA
Other Psychosis	5	NA	NA

7.3 Methods

7.3.1 *Neuroimaging and brain volumes*

The full details of the MRI pre-processing and volume extraction were described in chapter 2 'Aims and Methods'.

7.3.2 *Genotyping*

The method has been described in Chapter 3. Briefly, the DNA was obtained from blood or cheek swab samples. The DNA extraction method was the same for all subjects from the different studies. The summary of the genotyping methods used was fully described in Chapter 2: Aims and Methods.

7.3.3 *Candidate gene selection*

As noted earlier, I selected a number of candidate genes based on the most consistent finding on candidate genes for psychosis and brain volumes. Among these, I selected: Catechol-methyl-transferase -(COMT), Brain-Derived-Neurotrophic-Factor (BDNF), Neuregulin1 (NRG1) and Dysbinding (DTNBP1), as described in the Chapter 1: Introduction. Since the sample is composed of a combination of three different studies, I explored the role of the three SNPs that have been consistently genotyped across the three studies. These were: COMTval/met polymorphism (rs4680), BDNFval/met polymorphism (rs6265) and SNP8NRG243177 (rs6994992). Additionally, from the original MRI sample, I selected for this study four other SNPs. These were: SNP8NRG221533, SNP8NRG241930), DTNBP1P1757 (rs2005976) and DTNBP1P1320 (rs760761), consistently in the Maudsley Family and Twin Studies but not in the AESOP sample.

7.3.4 *Genome-Wide Association Study: GWAS*

In order to assess brain volume changes influenced by genetic factors, I also analyzed genetic data derived from the GWAS project carried out in the Department of Psychosis Studies, at the Institute of Psychiatry – see Chapter

2 Methods. I selected specific candidate genes based on a literature research, according to two criteria:

1) SNPs that have been described as candidate genes by playing a role in brain morphometry or white matter integrity in psychosis, which therefore might indirectly influence brain volume changes reported in psychosis:

DTNBP1_rs1047631
DTNBP1_rs875462
NTRK2_rs10868219
RELN_rs7341475

2) SNPs that have been described to directly influence brain volume:
MCPH1_rs2305022
MCPH1_rs930557
MCPH1_rs1057090
MCPH1_rs2912016
ASPM_rs3762271
OLIG2_rs1059004

3) Finally, I evaluated the effects on brain volumes of three genes that have been recently described by genome-wide association studies to affect brain volume in healthy individuals:

SBNO1_rs7980687
HMGA2_rs1042725
CRHR1_rs11655470

A number of SNPs that have been described in the literature to influence brain volume were not available in our GWAS sample. Therefore, I identified a proxy SNP for those SNPs and I described the results of this selection in Chapter 2: Aim and Methods.

7.4 Statistical analysis

The allele frequency and the deviations from Hardy-Weinberg equilibrium were assessed by chi-square tests using SPSS version 18 for Microsoft Windows (SPSS Inc, USA). For the statistical analysis of the genetic influence in brain volumes, I used both packages Stata version 10 (StataCorp LP, USA) and SPSS version 18 for Microsoft Windows (SPSS Inc., USA). The effect of candidate genes on brain volumes was examined using linear mixed models fitted with maximum likelihood methods. This method has been previously as described by Dutt et al (2009). Correlations between members of the same family were accounted for by including random intercepts for families, which is needed to maintain correct type 1 error rates. The dependent variables were grey, white and whole brain volume, while genotypes were entered as the main independent variables. In addition, all analyses were adjusted for the fixed effects of clinical group (patients, relatives and control). I also assessed genotype effect in brain volumes between patients and healthy volunteers using a linear regression analysis model with brain tissue volumes as the dependent variable. For these analyses the other additional independent variables were, age, sex and height.

7.5 Results

A total of 387 subjects were genotyped. However, from the genotyping obtained from the GWAS, only 149 passed Quality Control. The allele frequency for each group is summarized in the complementary table 1 and can be found in the Complementary Tables Section.

As described in chapter 2: Methods, 7 SNPs were genotyped either in the SGDP or by external companies focusing on a single SNP. COMTval/met polymorphism (rs4680), BDNFval/met polymorphism (rs6265) and SNP8NRG243177 (rs6994992) genotyping included subjects from the AESOP study, the Family Maudsley Study and the Maudsley Twin Study and thus the final genotyping data was obtained from more than 300 subjects. However,

when the sample was reduced for the SNPs genotyped in the Maudsley Family and Twin studies (SNP8NRG221533, SNP8NRG241930), DTNBP1P1757-rs2005976 and DTNBP1P1320-rs760761) the number of genotyped samples was reduced to around 230. In each genotyped SNP carried out in a smaller scale (i.e. locally or by external companies and no GWAS), there was a variation in sample sizes from the original 387 included subjects in sample size (complimentary tables and table Table 7.3). This variance is usually expected as a consequence of genotyping problems due to a number of factors such as biochemical artefacts and equipment factors i.e.Taq polymerase errors or low quality reagents; sample quality factor i.e. low quality or quantity of DNA and, human factor i.e. sample manipulation and confusion between samples (for example, mislabeling or tube mixing) (Pompanon et al., 2005).

The remaining 13 SNPs (DTNBP1_rs1047631, DTNBP1_rs875462, NTRK2_rs10868219, RELN_rs7341475, MCPH1_rs2305022, MCPH1_rs930557, MCPH1_rs1057090, MCPH1_rs2912016, ASPM_rs3762271, OLIG2_rs1059004, SBNO1_rs7980687, HMGA2_rs1042725, CRHR1_rs11655470) that I included in my thesis were extracted from GWAS data. Although GWAS data is less sensitive to human error than smaller scale genotyping, it is still greatly influenced by DNA low quality or quantity as well as population structures (Cardon and Palmer, 2003; Turner at al., 2011) and the Wellcome genotyping Centre has particularly stringent standards . These factors have played an important role in my study as the DNA samples had been collected over a period of up to 15 years, the demographic information such as ethnicity was recorded by self-asserted ethnicity by participants in combination with information from FIGS. In addition, DNA samples have been used for this and other genotyping before. All these factors have influenced both the quality and quantity of DNA leading to a significant reduction in sample size after undergoing GWAS quality control

(QC). The final sample for the remaining SNPs was therefore around 100 subjects.

7.5.1 Genetic analysis results

Genotype frequencies between patients and healthy controls did not deviate from the Hardy–Weinberg equilibrium. Complementary table 2. Bi-allele frequencies among each group with calculated Hardy–Weinberg equilibrium

For the statistical analysis, I combined the genotypes for which frequencies were less than 10 in either of the groups. Therefore the following: NRG 221533; NRG 241930; DTNBP1_rs2005976, DTNBP1_rs6937379, OLIG2_rs762178, SBNO1_rs12322888, NTRK2_rs10868219, RELN_rs7341475, MCPH1_rs2440416, MCPH1_rs2912057, MCPH1_rs2959798, ASPM_rs1360558, HMGA2_rs1042725, CRHR1_rs11655470 and BDNF rs6265 had two categories (i.e. heterozygous of low frequency were combined with the homozygous).

7.5.2 Genotypes and brain volume

I first analyzed brain volumes according to the genotype. Grey matter volume (GMV), white matter volume (WMV) and whole brain volume (WBM) in each group and according genotyping are described in Tables 7.2 to 7.10. In the statistical analysis all brain volume analysed were adjusted for age, gender, protocol and height and group.

Table 7.2: Neuregulin (NRG) and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
NRG 221533				
Patient				
CC/CT	63	0.69 (.071)	0.43 (.057)	1.13 (.121)
TT	37	0.7 (.082)	0.43 (.07)	1.13 (.141)
Relative				
CC/CT	70	0.67 (.076)	0.42 (.06)	1.1 (.14)
TT	43	0.68(.072)	0.42 (.067)	1.1 (.137)
Control				
CC/CT	41	0.69 (.07)	0.43 (.048)	1.12 (.112)
TT	18	0.69 (.08	0.42 (.065)	1.14 (.142)
NRG 241930				
Patient				
GG	37	0.69 (.07)	0.42 (.054)	1.11 (.115)
GT/TT	58	0.7 (.08)	0.44 (.06)	1.15 (.136)
Relative				
GG	40	0.68 (.077)	0.42 (.056)	1.1 (.128)
GT/TT	64	0.67 (.078)	0.43 (.07)	1.11 (.148)
Control				
GG	18	0.7 (.076)	0.44 (.062)	1.14 (.133)
GT/TT	31	0.68 (.077)	0.42 (.051)	1.11 (.123)

Table 7.2 cont: Neuregulin (NRG) and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
NRG 243177				
Patient				
CC	32	0.71 (.08)	0.44 (.068)	1.16 (.138)
CT	56	0.7 (.074)	0.43 (.053)	1.15 (.118)
TT	21	0.67 (.075)	0.41 (.055)	1.08 (.125)
Relative				
CC	33	0.68 (.084)	0.41 (.06)	1.09 (.141)
CT	53	0.66 (.06)	0.42 (.062)	1.09 (.128)
TT	10	0.7 (.09)	0.44 (.077)	1.15 (.162)
Control				
CC	19	0.68 (.086)	0.42 (.07)	1.1 (.155)
CT	29	0.68 (.083)	0.43 (.05)	1.11 (.125)
TT	15	0.69 (.09)	0.42 (.055)	1.11 (.143)

Table 7.3: Dysbindin (DTNBP) SNPs and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
DTNBP1P1757				
Patient				
AA/AG	31	0.7 (.075)	0.45 (.05)	1.16 (.115)
GG	45	0.69 (.069)	0.42 (.063)	1.11 (.127)
Relative				
AA/AG	44	0.68 (.084)	0.43 (.066)	1.12 (.152)
GG	58	0.67 (.07)	0.42 (.06)	1.09 (.125)
Control				
AA/AG	14	0.69 (.082)	0.42 (.048)	1.06 (.127)
GG	29	0.69 (.068)	0.43 (.047)	1.12 (.109)
DTNBP1P1320 rs760761				
Patient				
CC	35	0.7 (.068)	0.43 (.064)	1.13 (.125)
CT/TT	28	0.7 (.074)	0.45 (.052)	1.16 (.116)
Relative				
CC	53	0.67 (.074)	0.42 (.06)	1.09 (.129)
CT/TT	33	0.68 (.078)	0.44 (.064)	1.13 (.15)
Control				
CC	24	0.69 (.07)	0.42 (.052)	1.11 (.117)
CT/TT	15	0.68 (.07)	0.42 (.044)	1.09 (.113)

Table 7.3 cont: Dysbindin (DTNBP) SNPs and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
DTNBP1_rs6937379				
Patient				
CC/CT	29	0.7 (.08)	0.4 (.05)	1.12 (.13)
TT	32	0.7 (.062)	0.42 (.04)	1.1 (.104)
Relative				
CC/CT	26	0.68 (.08)	0.42 (.06)	1.1 (.13)
TT	37	0.68 (.084)	0.42 (.06)	1.1 (.14)
Control				
CC/CT	21	0.7(.08)	0.44 (.045)	1.14 (.13)
TT	6	0.6 (.07)	0.4 (.054)	1.02 (.122)
DTNBP1_rs1047631				
Patient				
CC/CT	13	0.71(.08)	0.43 (.044)	1.14 (.11)
TT	48	0.69 (.066	0.41 (.054)	1,1 (.11)
Relative				
CC/CT	15	0.7 (.085)	0.43 (.06)	1.13 (.14)
TT	48	0.68 (.081)	0.42 (.056)	1.1 (.13)
Control				
CC/CT	7	0.71 (.088)	0.45 (.05)	1.16 (.13)
TT	20	0.67 (.09)	0.42 (.049)	1.1 (.13)

Table 7.4: BDNF and COMT SNPs and brain volume distributions in groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
BDNF rs6265				
Patient				
AA/AG	39	0.69 (.06)	0.43 (.057)	1.12 (.104)
GG	94	0.7 (.083)	0.43 (.061)	1.14 (.136)
Relative				
AA/AG	32	0.69 (0.85)	0.43 (.07)	1.11 (.151)
GG	82	0.67 (.073)	0.42 (.06)	1.1 (.133)
Control				
AA/AG	26	0.68 (.09)	0.44 (.06)	1.12 (.145)
GG	54	0.69 (.08)	0.42 (.05)	1.11 (.125)
COMT rs4680				
Patient				
AA	37	0.69 (.074)	0.42 (.064)	1.11 (.131)
AG	62	0.7 (.081)	0.43 (.056)	1.13 (.127)
GG	32	0.72 (.071)	0.45 (.057)	1.16 (.119)
Relative				
AA	24	0.69 (.071)	0.43 (.06)	1.13 (.121)
AG	58	0.67 (.077)	0.41 (.06)	1.08 (.132)
GG	30	0.67 (.08)	0.43 (.07)	1.11 (.16)
Control				
AA	23	0.68 (.056)	???	???
AG	39	0.68 (.09)	0.43 (.06)	1.11 (.145)
GG	17	0.7 (.097)	0.44 (.052)	1.14 (.145)

Table 7.5: NTRK2 and brain volume distributions in groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
NTRK2_rs10868219				
Patient				
CC/CT	32	0.7 (.07)	0.42 (.047)	1.12 (.11)
TT	27	0.69 (.072)	0.41 (.06)	1.1 (.12)
Relative				
CC/CT	35	0.69 (.088)	0.42 (.058)	1.11 (.14)
TT	28	0.68 (.073)	0.42 (.057)	1.1 (.12)
Control				
CC/CT	11	0.69 (.074)	0.43 (.044)	1.12 (.11)
TT	15	0.69 (.098)	0.43 (.056)	1.12 (.15)

Table 7.6: RELN and OLIG2 SNPs and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
RELN_rs7341475				
Patient				
AA/AG	16	0.68 (.062)	0.41 (.06)	1.1 (.11)
GG	45	0.7 (.072)	0.42 (.05)	1.1 (.11)
Relative				
AA/AG	17	0.67 (.075)	0.42 (.062)	1.1 (.13)
GG	46	0.69 (.084)	0.42 (.056)	1.1 (.13)
Control				
AA/AG	9	0.68 (.09)	0.43 (.046)	1.12 (.13)
GG	18	0.68 (.09)	0.43 (.052)	1.1 (.13)
OLIG2_rs762178				
Patient				
CC	35	0.68 (.072)	0.4 (.054)	1.1 (.11)
CT/TT	25	0.69 (.067)	0.42 (.051)	1.12 (.12)
Relative				
CC	44	0.7 (.08)	0.41 (.048)	1.07 (.12)
CT/TT	14	0.66 (.08)	0.43 (.06)	1.13 (.13)
Control				
CC	19	0.66 (.089)	0.44 (.032)	1.17 (.1)
CT/TT	8	0.73 (.07)	0.42 (.055)	1.1 (.14)

Table 7.7: Microcephalin (MCPH1) SNPs and brain volume distributions between groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
MCPH1_rs2305022				
Patient				
AA	35	0.68 (.066)	0.41 (.053)	1.1 (.11)
AC/CC	26	0.7 (.074)	0.43 (.05)	1.13 (.11)
Relative				
AA	40	0.69 (.07)	0.42 (.057)	1.1 (.12)
AC/CC	23	0.68 (.09)	0.42 (.06)	1.1 (.15)
Control				
AA	13	0.68 (.09)	0.42 (.054)	1.1 (.14)
AC/CC	14	0.68 (.086)	0.43 (.047)	1.1 (.12)
MCPH1 _rs2440416				
Patient				
CC	37	0.69 (.069)	0.41 (.05)	1.1 (.11)
CG/GG	24	0.7 (.074)	0.43 (.053)	1.12 (.12)
Relative				
CC	41	0.67 (.073)	0.41 (.062)	1.1 (.12)
CG/GG	22	0.7 (.09)	0.43 (.054)	1.14 (.15)
Control				
CC	14	0.68 (.095)	0.42 (.051)	1.1 (.14)
CG/GG	12	0.68 (.092)	0.43 (.052)	1.1 (.13)

Table 7.7 cont: Microcephalin (MCPH 1) SNPs and brain volume distributions between groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
MCPH1 _rs2912057				
Patient				
AA/AG	36	0.69 (.072)	0.41 (.055)	1.1 (.12)
GG	23	0.7 (.067)	0.42 (.047)	1.13 (.1)
Relative				
AA/AG	39	0.69 (.086)	0.43 (.062)	1.12 (.14)
GG	23	0.68 (.075)	0.42 (.05)	1.1 (.12)
Control				
AA/AG	17	0.69 (.099)	0.44 (.052)	1.12 (.14)
GG	10	0.67 (.072)	0.42 (.044)	1.1 (.11)
MCPH1 _rs2959798				
Patient				
CC/CT	35	0.69 (.07)	0.41 (.054)	1.1 (.11)
TT	26	0.69 (.069)	0.41 (.051)	1.1 (.11)
Relative				
CC/CT	37	0.69 (.08)	0.43 (.058)	1.13 (.13)
TT	26	0.67 (.78)	0.41 (.053)	1.08 (.12)
Control				
CC/CT	14	0.69 (.097)	0.44 (.056)	1.13 (.15)
TT	12	0.68 (.077)	0.42 (.044)	1.1 (.11)

Table 7.8: ASPM and brain volume distributions between groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
ASPM _rs1360558				
Patient				
CC/CT	49	0.68 (0.69)	0.41 (.054)	1.1 (.11)
TT	12	0.72 (.064)	0.43 (.042)	1.16 (.1)
Relative				
CC/CT	51	0.68 (.075)	0.42 (.056)	1.11 (.12)
TT	12	0.69 (.1)	0.41 (.065)	1.1 (.17)
Control				
CC/CT	18	0.69 (.07)	0.43 (.042)	1.13 (.1)
TT	9	0.66 (.12)	0.42 (.064)	1.08 (.18)

Table 7.9:SBNO1and HMGA2 CHRH1 SNPs and brain volume distributions among groups

	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
SBNO1 _rs12322888				
Patient				
AA/AG	23	0.7 (.073)	0.42 (.056)	1.13 (.12)
GG	38	0.68 (.067)	0.41 (.05)	1.1 (.11)
Relative				
AA/AG	26	0.7 (.09)	0.43 (.067)	1.13 (.15)
GG	37	0.67 (.071)	0.41 (.049)	1.1 (.11)
Control				
AA/AG	8	0.69 (.11)	0.44 (.063)	1.13 (.17)
GG	19	0.68 (.078)	0.42 (.042)	1.1 (.11)
HMGA2_rs1042725				
Patient				
CC	19	0.68 (.07)	0.41 (.056)	1.1 (.12)
CT/TT	41	0.7 (.067)	0.42 (.051)	1.12 (.11)
Relative				
CC	17	0.69 (.963)	0.43 (.051)	1.12 (.11)
CT/TT	46	0.68 (.088)	0.42 (.059)	1.1 (.14)
Control				
CC	12	0.66 (.084)	0.42 (.052)	1.08 (.13)
CT/TT	14	0.7 (.097)	0.44 (.05)	1.14 (.13)

Table 7.10:CHRH1 SNP and brain volume distributions among groups

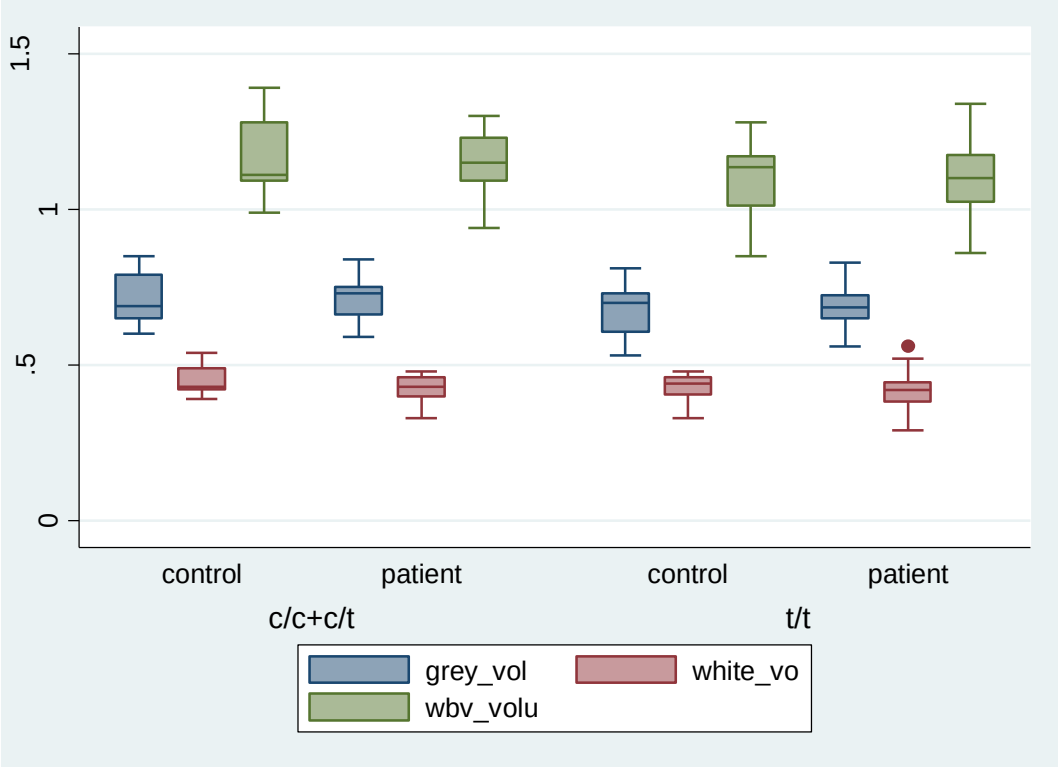
	n	Mean GMV in Litres (S.D.)	Mean WMV in Litres (S.D.)	Mean WBV in Litres (S.D.)
CRHR1_rs11655470				
Patient				
AA/AG	40	0.69 (.078)	0.42 (.052)	1.1 (.12)
GG	20	0.69 (.052)	0.41 (.054)	1.1 (.1)
Relative				
AA/AG	44	0.69 (.076)	0.42 (.057)	1.1 (.12)
GG	19	0.68 (.095)	0.41 (.058)	1.1 (.14)
Control				
AA/AG	14	0.7 (.11)	0.43 (.056)	1.13 (.16)
GG	13	0.67 (.062)	0.42 (.043)	1.1 (.09)

7.6 Genetic analysis

7.6.1 DTNBP1_rs1047631 and brain volumes

The patients group homozygous for thymidine (i.e. TT) showed smaller volumes than healthy volunteers homozygous TT in the three brain volumes measured. This was statistically significant as the estimated mean difference in litres for grey matter was – 0.025 (95% CI= -.45 to -.023; p=0.028), for white matter was -0.018 (95% CI=-.039 to -.0009; p=0.038) and for whole brain volume, it was -0.043 (95% CI=-.08 to -.007; p=0.016). No differences were found in other group comparisons.

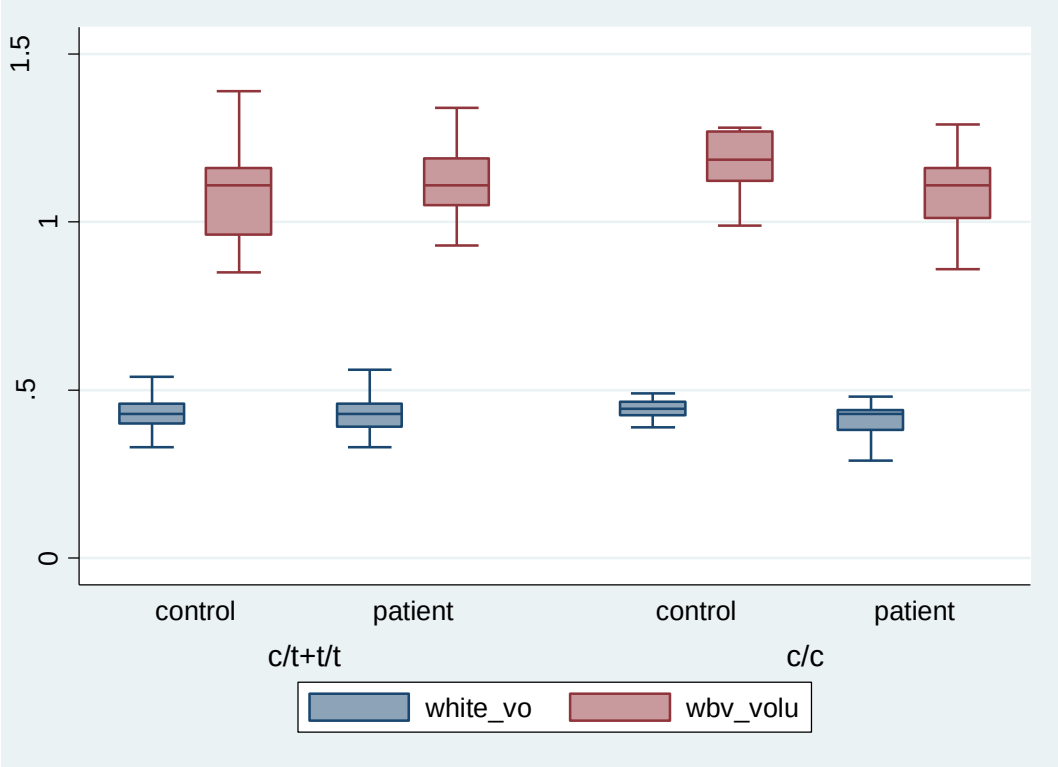
Graph 7.1: DTNBP1_rs1047631 and grey, white and whole matter volumes



7.6.2 OLIG2_rs762178 and brain volumes

OLIG2_rs762178 gene did not correlate with grey matter volume in any of the assessed groups. However, when I compared homozygous for cytosine (i.e. CC) between patients and healthy control groups, white matter volume in patients showed smaller white matter volume than healthy controls with an estimated mean difference in litres of -0.017 (95% CI=-.03 to -.004; p= 0.012) as well as smaller whole brain volume with estimated mean difference in litres of -0.035 (95% CI=-.065 to -.0047; p=0.023). Since OLIG2 is involved in the development of white matter cells, it was expected that its effect was more significant in white matter volume. No differences were found in other group comparisons

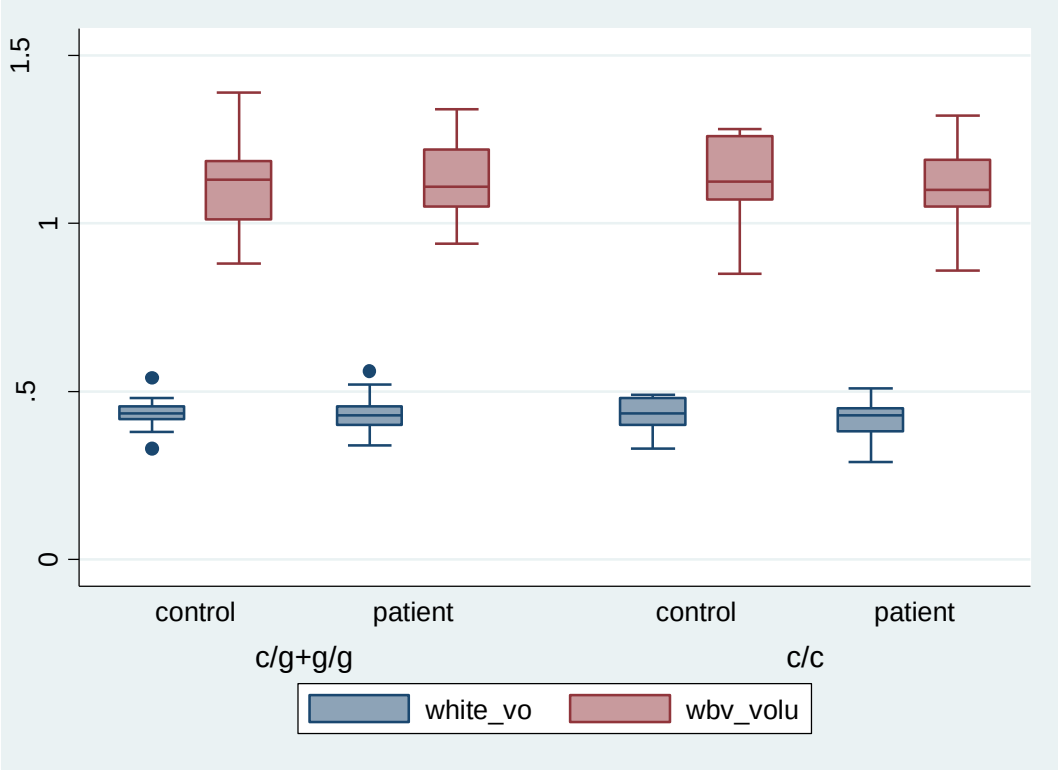
Graph 7.2: OLIG2 and white and whole matter volumes



7.6.3 MCPH_rs930557 and brain volumes

No significant differences in grey matter volumes were observed between genotypes. However, in the patients group those homozygous for cytosine (CC) showed significantly smaller white matter than healthy volunteers homozygous for cytosine (CC) with a estimated mean difference in litres of -0.015 (95% CI= -.029 to -.0009; p=0.036 and, at trend level smaller whole brain volume with an estimated mean difference in litres -0.027 (95% CI= -.058 to .003; p=0.078). No differences were found in other group comparisons

Graph 7.3: MCPHrs930557 and white and whole matter volumes



Although the differences discussed above which were nominally significant, no association would survive Bonferroni corrections for 20 independent tests (Bonferroni corrected p-value should be 0.05/20 ie 0.0025). In addition, given the small sample size and the multiple testing corrections needed for this analysis, I was not able to compare the two diagnostic groups, schizophrenia and bipolar disorder separately. The combined analyses including the three groups did not showed statistical significant difference.

7.6.4 Other genes

With regards to the other 17 SNPs included in the analyses, there were no significant associations between variations in any of them against either of the brain matter volumes measured.

Chapter 8: Discussion

In this study I examined brain matter volumes as plausible endophenotypes for psychosis. For this, I aimed to explore the potential role that genes involved in psychosis and in neurodevelopment may play in brain volumes in a large sample of patients with psychosis, their relatives, and a group of healthy individuals. Since the brain volumes had been acquired with MRI scans that used different protocols, the first step of this analysis was to design and carry out a calibration study to evaluate the compatibility of these MRI protocols, which although different, were all obtained on the same scanner. The results of this calibration study showed a high intra-class correlation in volumes between the protocols used (above 0.9). Therefore, I was able to combine MRI data from the samples from the three original studies. This approach enabled me to increase the statistical power necessary to evaluate total grey and white matter volume, as well as the whole brain volume, in relation to diagnosis risk and familiality, and assess them as potential endophenotypes. After this first step, I could proceed to compare patients with psychosis with healthy controls. In particular, I assessed grey and white matter volume as well as whole brain volume differences between patients with psychosis and healthy volunteers. I then compared these volumes in these two groups with those of the relatives of subjects with psychosis, to estimate brain volume differences as well as familiality. In the final step, I explored the genetic influence of certain SNPs on brain volumes.

8.1 Case control brain volume

8.1.1 Patients vs healthy volunteers*:*

In the case-control analysis, I first explored the question of to what extend psychotic disorders can be considered as different expression of one disorder, where Schizophrenia and Bipolar disorder would represent the extremes

along a continuum of psychosis. Therefore, I compared patients group (schizophrenia and bipolar disorder together) against the healthy volunteers.

8.1.1.1 *Patients vs healthy volunteers: Grey matter volume*

I found that the comparison of the two disorders, Schizophrenia and Bipolar, pooled together as one disorder group against healthy controls, showed no statistically significant differences in total grey matter volume. In a previous extensive review (Shenton et al., 2001) and several meta-analytical studies, which evaluated larger samples, grey matter volume reductions have been found in both chronic and first-episode schizophrenia patients (Vita et al., 2006; Ellison-Wright et al., 2010), in bipolar disorders (Strakowski et al., 2005) and in both, schizophrenia and bipolar disorder (Arnone et al., 2009; De Peri et al., 2012) against healthy volunteers. However, the evidence for grey matter reduction in bipolar disorder in comparison to healthy volunteers has not been consistently found, as shown in MacDonald and colleagues (2004) meta-analysis. In addition, McDonald and colleagues have found reductions in grey matter volumes in patients with schizophrenia but not in bipolar disorder when compared to healthy volunteers (McDonald et al., 2005). However, all these studies compared each psychosis diagnosis separately against the healthy volunteers, rather than as one single patient group as I did in my analysis. Therefore, this methodological difference between those studies and mine might have contribited to the lack of difference in grey matter volumes identified in my analysis.

However, a recent multimodal meta-analysis that analyzed grey matter volumes in patients with schizophrenia and bipolar disorder together against healthy volunteers found significant total grey matter reductions in patients relative to the healthy volunteers, and interestingly, this was influenced by the use of antipsychotics (Radua et al., 2012). Although the methodology was similar to mine, in Radua's study patients were all at their illness first onset while my study included patients who were in both, their first onset and

chronic stages of the illness. Therefore, my sample is clinically and, may be demographically, more heterogeneous. Moreover, including patients at different illness stages might give rise to more variability and less accuracy in the results and thus, contribute to the lack of positive findings in this type of analysis. Another possible explanation for the absence of differences in grey matter volume that I found is that the first episode patients and some of the healthy controls I used were recruited as part of the AESOP study. For this study, the participants were recuited using an epidemiological approach, from the same catchment area. In previous studies that have reported on patients with first-episode schizophrenia or psychosis, the referrals came from university clinics, referral centres and in-patient samples, which attract subjects not necessarily representative of first-episode psychosis in general (Morgan et al., 2007). It is possible that my sample included subjects with a less severe illness than those used in previous studies. Furthermore, the healthy controls were also intended to represent the general population and were not just selected from hospital or university staff. Consistently with my findings, even the comparison between patients and controls in the original AESOP study did not reveal any difference in global volumes between these groups (Morgan et al, 2007). This highlights the importance of selecting epidemiologically representative comparison groups when comparison measures such as brain volumes that can be affected by socio-environmental factors.

8.1.1.2 *Patients vs healthy volunteers: White matter volume*

In my study I did not find a significant difference in white matter volumes between the combined patient group (i.e. schizophrenia and bipolar disorder together) and healthy controls. There is scarce evidence of global white matter volume differences when patients with different psychoses are compared as a group to healthy volunteers. Previous studies with a combined sample of first episode of psychosis did not report a comparison of global white matter volumes, but rahter regional white matter reductions in several

temporal lobe areas in patients in comparison to healthy volunteers (Price et al., 2010). However, a recent study that included patients at their first episode psychosis using VBM found no differences when patients as a single group were compared to healthy controls (Colombo et al., 2012). This evidence is consistent with the negative findings of my analysis of white matter volumes, in the comparison between patients groups as one group and controls. On the other hand, another very recent study showed that both patients with schizophrenia and bipolar disorder had smaller total white matter volume than healthy controls (De Peri at al., 2012). Previous studies that evaluated white matter volumes in these disorders separately but against the same control group have reported contradictory findings. Some studies have found white matter reductions in both schizophrenia (Buchanan et al., 1998, Wright et al., 2000, De Peri et al., 2012) and bipolar disorder (De Peri et al., 2012), while others found no difference in patients with schizophrenia (Ananth et al 2002) or bipolar disorder (McDonald et al., 2005) in comparison to healthy volunteers. However, similarly to studies in grey matter volume, most of these studies did not analyze the patient's group sample together but as two separate groups against healthy volunteers. As mentioned above for the grey matter, the lack of findings in my sample might also be explained by the inclusion of patients and controls recruited with an epidemiological approach, hence possibly less sick (for patients) and not selected (Morgan et al., 2007).

8.1.1.3 *Patients vs healthy volunteers: Whole brain volume*

In my study I did not find a significant difference between the patients as a whole and the healthy controls. As for total grey and white matter volume, the comparison of whole brain matter volume between patients with psychosis analyzed as only one disease entity (i.e. schizophrenia and bipolar disorder together) and healthy volunteers, is not usually reported. Previous studies have consistently reported reductions in whole brain matter volume between patients with established schizophrenia and healthy volunteers, as suggested in several meta-analyses (Wright et al., 2000, Vita et al., 2006; Ellison-Wright

et al., 2010). On the other hand, no differences in whole brain matter volume have been reported between bipolar disorder patients and healthy volunteers (Hoge et al., 1999; McDonald et al., 2004; Kempton 2008). However, more recently, studies have shown reductions of whole brain matter volume in both, schizophrenia and bipolar disorder, when compared to healthy volunteers (Arnone et al., 2009, De Peri et al., 2012).

In conclusion, due to the dichotomous classification of psychosis, most studies have compared schizophrenia and bipolar disorder separately against the same healthy control group, with only few studies comparing both illnesses together versus a control group. There is evidence from symptom factorial analyses that schizophrenia and bipolar disorder might be the expression of different extremes of a continuous illness (Dikeos et al 2006, Demjaha et al., 2009), sharing epidemiological as well as neurobiological risk factors (Murray et al., 2004; Craddock et al., 2005b, Arajarvi et al., 2006), and a genetic overlap (Bramon and Sham, 2001; Sklar et al., 2002; Craddock et al, 2005a; Lake, 2007), providing a potential endophenotype overlap (Arts et al 2008, Ivleva et al., 2010). Interestingly, in my study the combination of these two diagnostic categories into one did not highlight any significant difference in brain volumes in comparison to healthy volunteers.

These findings can be the result of a lack of power to detect subtle brain volume differences between patients and healthy volunteers. In addition, there is increasing evidence that the brain changes in psychosis are distributed in nature, and therefore global brain tissue volumes may not represent the best candidate endophenotype for these disorders (Mourao-Miranda et al, 2012). Moreover, the additional morphometric heterogeneity that derives from the combination of schizophrenia and bipolar disorder as a single group might explain the lack of significant differences in the brain measures evaluated. Moreover, the use of medication might have also played a role in these findings. There is evidence that psychoactive medications, including

antipsychotics (Navari & Dazzan, 2009; Lieberman et al., 2005) and lithium (Moore et al., 2000; Smieskova et al., 2009; Moncrieff and Leo, 2010; Hallahan et al., 2011) might affect grey and white matter as well as whole brain volume volumes.

8.1.2 Diagnostic Groups

The second case-control analysis I conducted followed the dichotomous theory of psychosis. In this analysis I sought to define a possible endophenotype that is illness specific. For this, the patient group was split into two diagnostic groups: schizophrenia and bipolar disorder. I decided to exclude patients with a diagnosis of other psychoses to eliminate further heterogeneity in the sample. Therefore, I centred the analysis on the comparison of patients with schizophrenia and patients with bipolar disorder, comparing each group to the same healthy volunteer group as well as against each other.

8.1.2.1 Grey matter differences

8.1.2.1.1 *Schizophrenia vs Healthy volunteers*

In the grey matter volume analysis, patients with schizophrenia showed statistically significant smaller grey matter volume when compared to the healthy volunteers. This difference in grey matter between patients with schizophrenia and healthy controls is consistent with findings from previous studies (Shenton et al., 2001; McDonald et al., 2005; Bose el al., 2009) and meta-analyses (Ward, 1996; Wright et al., 2000; Fornito et al., 2009; Haijma et al., 2012). Moreover, this deficit has been also reported in a recent study using VBM methods of analysis (Yuksel et al., 2012). Furthermore, and relevant to my study, recent meta-analyses show that deficits in grey matter volumes are more marked in schizophrenia than in bipolar disorders (Ellison-Wright and Bullmore, 2010; De Peri et al., 2012).

When comparing structural imaging data of patients with schizophrenia with those of normal controls, the more consistent evidence has been that of a reduction of whole brain volume, as well as of total grey and white matter volumes, in patients, as summarized by a recent meta-analysis (Olabi et al., 2011). Interestingly, these reductions have been reported to be progressive in nature and proposed as an indicator of poor prognosis (Andreasen et al., 2011). Moreover, the evidence of progressive grey matter loss has also been considered to suggest that the illness may have a degenerative component (Rund, 2009; Gupta & Kulhara, 2011). However, this has been difficult to prove, and there is indeed evidence to suggest that the brain changes observed in schizophrenia may result from a neurodevelopmental abnormality in this illness (Weinberger 1986, 1987; Murray & Lewis1987; Van Os & Kapur, 2009; Gupta & Kulhara, 2011; Owen et al., 2011). Furthermore, the progressive changes seen in psychosis have also been related to the prolonged exposure to antipsychotics in later illness stages. For example, a recent longitudinal study by Ho and colleagues (2011) has prospectively evaluated a large sample of patients with recent onset schizophrenia and evaluated brain volume changes overtime in relation to the use antipsychotic medication. The authors found that more use of antipsychotic medication was associated with greater brain volume loss in both white and grey matter volumes. This effect remained significant after correcting for illness severity and substance misuse. Although in my study I included patients at their first and more chronic stages of schizophrenia, the cross-sectional comparison of brain volumes in the two stages was thought to be inappropriate. To assess brain volumes progression or changes over time, the same sample would need to be assessed at two or more times. However, as a proxy measure for time, I assessed the impact of illness duration on brain volumes and, although only at trend level, the results go in line with this evidence. In fact, I found that a longer duration of illness was correlated with smaller whole brain volume in patients with schizophrenia. It is important to consider that in this study the white matter volume in patients with schizophrenia was significantly larger

than in the other two groups (patients with bipolar disorder and healthy volunteers). Therefore, this finding may be interpreted as indirect evidence that the association between longer illness duration and smaller whole brain volume was due to a reduction in grey matter in individuals with longer illness duration.

With regards to medication, there is evidence from animal models that antipsychotics (both haloperidol and olanzapine) medication might have a direct effect on brain structure, and hence cause some of the volume deficits found in schizophrenia (Vernon et al., 2011). However, an MRI study of patients with schizophrenia (and all psychoses) found slightly different results in grey matter reductions with different antipsychotics (Lieberman et al., 2005). For example, the authors found that the effects on grey matter reductions were related to haloperidol but not olanzapine. In addition, antipsychotics seem to act differently in different brain structures. For example, typical antipsychotics seem associated with basal ganglia volume increase (Scherck et al., 2006; Wright et al., 2000) but with cortical grey matter decrease (Scherck et al., 2006). In my study, 9 patients with schizophrenia were drug naïve, 25 were taking typical antipsychotics, 85 were taking atypical antipsychotic and 4 were on a combination of both at the time of the MRI. This heterogeneity of medication, as described in previous studies, might play a role in the results and highlights the importance of obtaining a detailed treatment history in neuroimaging studies.

Finally, my findings of grey matter reductions in patients with schizophrenia in comparison to healthy volunteers are consistent with those previous studies using also VBM and a multi-study neuroimaging design (Meda et al., 2008; Segall et al., 2009). Therefore, my findings lend further support to the application of VBM in multi-site/protocol studies.

8.1.2.1 2 *Bipolar disorder vs Healthy Volunteers*

Unlike schizophrenia, patients with bipolar disorder in my study showed no differences in grey matter volume when compared to healthy controls. The number of studies looking at volumetric changes in bipolar patients in comparison to healthy controls is significantly smaller than that of schizophrenia. Additionally, most studies looked at regional volumes rather than global volumes (Paerlson et al., 1997; Paerlson et al., 1999; Strakowsky et al. 1999, McDonald et al 2004; McDonald et al 2005). However, previous studies looking at segmented global volumes have reported contradictory findings, with some describing smaller grey matter volumes (Lim et al., 1999; Lopez-Larson et al., 2002; De Peri et al., 2012), and others finding no differences between patients with bipolar disorder and healthy volunteers (Zipursky et al., 1997; Brambilla et al., 2001; McDonald et al., 2005; Wilke et al 2004). Furthermore, De Peri and colleagues found that grey matter volume reductions seem to be less marked in patients with bipolar disorder than in schizophrenia when compared to healthy volunteers (De Peri et al., 2012). Therefore, the relative lack of loss of grey matter volume at first presentation, in combination with recent evidence that lithium might increase grey matter volume in patients taking lithium (Hallahan et al., 2011), might help explain the lack of differences in grey matter volume I found between patients with bipolar disorder and healthy volunteers. In my sample, 23 patients were taking lithuim at the time of the MRI, and all of these patients were in the chronic stages of the bipolar illness. Although this study was not designed to evaluate psychotropic drugs effects on brain volume, one could argue that at least some patients might have been taking lithuim for long enough to explain the less marked reduction of grey matter volume I observed in comparison to healthy volunteers.

8.1.2.1 3 *Schizophrenia vs Bipolar disorder*

There have been only few studies that have compared these two major psychotic illnesses. However, consistently with previous literature, patients

with schizophrenia in my study showed a statistically significant smaller grey matter volume when compared to bipolar patients (McDonald et al., 2005; Yuksel et al., 2012). In addition, most studies evaluating regional grey matter volumes have described signifficant reductions in grey matter in areas such as the insula (Kasai et al., 2003) in patients with schizophrenia in comparison to patients with bipolar disorder. Also, Kubicki and colleagues (2002) used a VBM approach and found statistically significant smaller medial temporal grey matter in schizophrenia compared with bipolar disorder. However, other studies have not found grey matter differences between bipolar disorder and schizophrenia patients in these medial temporal areas (Hirayasu et al., 1998) or in the insula (Kubicki et al., 2002).

A recent meta-analysis by Ellison-Wright and Bullmore (2010) showed a difference in grey matter between patients with bipolar disorder and patients with schizophrenia, when compared to healthy volunteers. While grey matter reductions in paralimbic regions were present in bipolar, grey matter reductions in schizophrenia were more widespread, involving limbic, neocortical structures and the paralimbic regions. Although this was a not a direct comparison between bipolar disorder and schizophrenia, the findings of more extensive areas of grey matter reductions in patients in schizophrenia than in patients with bipolar disorder could be consider indirect evidence of grey matter reductions in schizophrenia compared to bipolar disorder. Moreover, a more marked reduction in grey matter volume in schizophrenia than in bipolar disorder in comparison to healthy volunteers has been recently shown to be already present at illness onset in children and adolescent onset (Arango et al., 2012) and in adult onset (De Peri et al., 2012) schizophrenia. However, a meta-analysis that directly compared schizophrenia and bipolar disorder failed to find global grey matter differences between the two groups (Kempton 2008).

In my study I included patients in both chronic and early stages of their illness. Therefore, there might be a number of issues that could have played a role in

the results. For example, having a heterogeneous sample makes it more difficult to account for the effects of medication. It has been described that grey matter volume increases in bipolar disorder patients in comparison to healthy controls in association with the use of lithium (Hallahan et al., 2011). Moreover, there is also evidence that the use of antipsychotic medication is associated with smaller grey matter volumes (Lieberman et al., 2005; Moncrief & Leo 2010, Leung et al., 2011), and that this is more evident with the use of typical antipsychotics (Navari & Dazzan, 2009). Moreover, changing from typical to atypical antipsychotic may have an effect on regional volumes, such as for example those of the basal ganglia (Scherk et al., 2006). In my study, 9 patients with schizophrenia were drug naïve, 25 were taking typical antipsychotics, 85 were taking atypical antipsychotic, 4 were on a combination of both, and among patients with bipolar disorder 14 were taking antipsychotics and 23 were taking lithium at the time of the MRI. Therefore, although my findings are consistent with previous reports of grey matter differences between patients with bipolar disorder and patients with schizophrenia, these findings should be investigated further, further exploring of the role of medication in explaining brain volume differences between two groups which tend to be exposed to different kinds of psychotropic drugs.

8.1.2.2 White matter differences

8.1.2.2.1 *Schizophrenia vs Healthy Volunteers*

With respect to white matter volume, there is less evidence from existing literature on differences between patients with schizophrenia and healthy controls. The findings of structural white matter changes reported to date have been inconsistent. Previous studies have described reductions in white matter volume when comparing patients with schizophrenia and healthy controls (Sigmundsson et al., 2001; Paillere-Martinot et al., 2001). The reduction has been shown both in patients at their first episode as well as in those at more chronic stages (Whitford et al., 2007; Di et al., 2009; De Pieri et al., 2012).

However, some studies have failed to replicate these findings (Hulshoff Pol et al., 2004; Moreno et al., 2005). Additionally, most recent studies have put their emphasis on regional rather than global white matter changes, as also outlined in a recent meta-analysis (Di et al., 2009). Also, they have looked at white matter integrity, rather than volume, with sequences such as diffusion tensor imaging (DTI) (Lim at al., 1999). Still, DTI data show evidence of altered white matter integrity in schizophrenia (Lim at al., 1999; Kubicki et al., 2005; Bora et al., 2011). This evidence supports the hypothesis that there might be structural white matter volumetric changes in patients with schizophrenia, but that these may be difficult to identify in group comparisons that use VBM methods of analysis. In the current study, patients with schizophrenia presented with larger global white matter volume than controls. This finding is in contrast with those of a recent meta-analysis by Di et al (Di et al., 2009). Although this meta-analysis included studies of patients at first and chronic stages of illness, making it more similar to my sample, the authors commented on a number of technical limitations, suggesting that the results of the meta-analysis may have depended on the heterogeneity introduced by illness stage. For instance, they note that white matter concentration (WMC, from unmodulated data) or white matter volume (WMV, from modulated data) measurements were reported depending on whether the modulation step had been applied. Modulation is a step thought to be more appropriate when evaluating brain volumes, as this optional step in the pre-precessing pipeline aims to correct for deformations that might occur during spatial normalization (Good et al., 2001; Mechelli et al., 2005). Importantly, differences between modulated or unmodulated brain volume measurements have been demonstrated for grey matter measurements (Eckert et al., 2006). However, the theory applies to both white and grey matter (Good et al. 2001; Mechelli et al., 2005), and therefore methodological issues such as using modulated data might have impact white matter volume measure in my study. Another important point is that the smoothing kernel used across studies could also have affected VBM results and their comparability. So, although my study did

not found a reduction in white matter volume in patients (but rather an increase), it has the advantage that all neuroimaging data were processed by one investigator only, with the same method, and applying the same kernel giving more reliable results. This evidence suggests that white matter, for its very nature, may be particularly susceptible to the limitations of VBM approaches, and that potential alterations of this tissue need further investigation, possibly with methods more suited to white matter such as DTI.

8.1.2.2.2 *Bipolar Disorder vs Healthy volunteers*

In this study I found that patients with bipolar disorder have smaller white matter voulmes than healthy volunteers. The literature in structural white matter changes in bipolar disorders brings contradictory evidence. Most studies have described no volumetric differences in white matter volumes in bipolar disorder patients in comparison to healthy individuals (Zipursky et al., 1997; Brambilla et al., 2001; Lim et al., 1999; Lopez-Larson et al., 2002). However, more recently, some studies have described smaller white matter volume in patients with bipolar disorder in comparison to healthy volunteers (Kieseppa et al., 2003; McDonald et al., 2005; Vita et al, 2009). In addition, reduced white matter volume in patients with shizophrenia and bipolar disorder in comparison to healthy volunteers have also been described in a recent meta-analysis (De Peri et al., 2012). Moreover, the authors of this meta-analysis concluded that smaller white matter volumes in patients with psychosis seem to be more characteristic of bipolar disorder than schizophrenia. The suggestion that white matter alterations may be more specific to bipolar disorders seems supported by previous studies finding an increase in white matter hyperintensities in patients with bipolar disorder than in healthy volunteers (Figiel et al., 1991; Aylward et al., 1994; Dupont et al., 1995; McDonald et al., 1999). However, as for white matter volume, others have failed to find a significant association (Lewine et al., 1995; Persaud et al., 1997). White matter hyperintesnities have been understood as a marker of ischaemic lesions, as shown by neuropathological inspection (Awad et al.,

1986; Fazekas et al., 1993). Althought these hyperintensities do not seem to be specific to bipolar disorder, as they are strongly correlated with age in non-psychiatric populations (Schmidt et al., 1999), several meta-analysis have found strong evidence for an association between increased hyperintensity lesions and bipolar disorder (Altshuler et al., 1995; Videbech et al., 1997; Kempton et al., 2008; Beyer et al., 2009). Although in my study I did not assess white matter hyperintensities, evidence of increased white matter hyperintensities in patients with bipolar and the evidence that they are likely to reflect an ischaemic damage possible leading to volume reductions, may indirectly support my finding of smaller white matter volume in patients with bipolar disorder.

Finally, previous studies have described that while lithuim does not seem to have an effect on segmented white matter volume, antipsychotics may have a negative correlation with this measure (Kieseppa et al., 2003; Ho et al., 2011). In my sample, 14 patients with bipolar disorder were taking antipsychotics and 23 were taking lithium at the time of the MRI. It is possible that some of my findings could have been related to the exposure to these psychotropic medications.

8.1.2.2.3 *Schizophrenia vs Bipolar disorder*

In the current study, patients with bipolar disorder showed smaller whole white matter volumes than patients with schizophrenia. This finding is novel, as for both illnesses white matter volumes have been reported as reduced in comparison to healthy controls (MCDonald et al., 2005), but not between patients with schizophrenia and patients with bipolar disorder. There is less evidence on differences in white matter volumes between the two as suggested in a recent meta-analysis by Kempton et. al. (2008). The authors included only 3 studies reporting global white matter volume (with a total of 50 bipolar and 78 schizophrenia patients) and found no differences in global white matter volume between schizophrenia and bipolar disorder.

As described above, white matter hyperintensities seem to be related to small brain injury, possibly leading to volume reductions (Awad et al., 1986; Fazekas et al., 1993), and these have been more frequently described in patients with bipolar disorder (Altshuler et al., 1995; Videbech et al., 1997; Kempton et al., 2008; Beyer et al., 2009; Emsell and McDonald, 2009) and their relatives (Hajek et al., 2005) than in healthy volunteers. Interestingly, a study by Rivkin et al. (2000) found that even when the frequency of white matter hyperintensities was compared in young and elderly people with shizophrenia and healthy volunteers, there were no differences. This suggests again that white matter volume might be more extensevely affected in bipolar disorder than in schizophrenia.

Finally, the lack of structural neuroimaging evidence for white matter differences between schizophrenia and bipolar disorder may be related to technological advances in the evaluaiton of white matter. Most studies evaluating white matter have been focusing on white matter integity as measured by tractography or DTI. These more modern approaches are preferred to structural neuroimaging in psychosis, as they are more likely to provide information more closely related to the integrity and function of this tissue. In addition, they might be more able to distinguish diffent neuroconnectivity patterns related to the different clinical presentations of schizophrenia and bipolar disorder (Cui et al., 2011).

8.1.2.3 Whole brain volume differences

8.1.2.3.1 *Schizophrenia vs Healthy Volunteers*

The comparison of whole brain matter volume showed a statistically significant difference between these two groups, with larger volumes in healthy controls than in patients with schizophrenia. This result is not surprising and is in line with previous studies of brain volumes using both regional and whole brain VBM methods of analysis (Wright et al., 2000,

Ellison-Wright et al., 2008; De Peri et al., 2012). Moreover, the reduction of whole brain volume in patients with schizophrenia in comparison to healthy volunteers seems to be present from the illness onset (Vita et al., 2006). The present study is a combination of first episode and chronic presentations of schizophrenia, similarly to those included in meta-analysis by Wright and colleagues (Wright et al., 2000). To observe such reductions in this patient group, one should assume that patients at both stages, onset and chronic, would present with whole brain volume reductions. Moreover, in my study, I found that a longer duration of illness was correlated with whole brain volume reduction in patients with schizophrenia. The evidence for whole brain volume change over time in patients with schizophrenia has been contradictory, with some studies describing progressive volume loss (DeLisis et al., 2004; Ho et al., 2003) and others not finding these changes (Steen et al., 2006; Whitworth et al., 2005). Interestingly, a recent meta-analysis seems to confirm evidence of a progressive reduction (Olabi et al., 2011). This finding supports the hypothesis that combining first onset with chronic patients increases the statistical power needed to detect brain volume differences. However, as mentioned above, it is important to assess to what extent these differences reflect a medication effect or whether they are a result of specific psychopathological mechanisms, or of a genetic predisposition, which may be addressed by more specifically designed studies.

8.1.2.3.2 *Bipolar Disorder vs Healthy Volunteers*

In this study I did not find any difference in whole brain volume in patients with bipolar disorder when compared to healthy controls. My results seem consistent with previous findings (Zirpursky et al., 1997; Brambilla et al., 2001). In addition, my results support the findings of three previous meta-analyses, which have found no differences between chronic patients with bipolar disorder and healthy controls (Hoge et al., 1999; McDonald et la., 2004, Kempton 2008), and with those of one meta-analysis of patients at their first episode of bipolar disorder (Vita et al., 2009). However, Arnone's meta-

analysis (2009) reported a reduction of whole brain volume in patients with bipolar disorder compared to healthy controls. The authors pointed out that the heterogeneity in sample characteristics and the methods used in the studies may have explained their findings. Although the present study should have enough statistical power to detect structural brain volume differences between patients with bipolar disorder and healthy controls (Friston et al., 1999), these might be very subtle and therefore, a larger sample might be needed. Arnone's meta-analysis included 661 participants with bipolar disorder and 723 healthy controls, carrying more statistical power than the one of my study and of previous meta-analyses (Hoge et al., 1999; McDonald et al., 2004). However, the meta-analysis by Kempton et al., (2008) also included a very large sample size and still failed to find statistically significant differences in whole brain volume between patients with bipolar disorder and healthy volunteers. Therefore, it is possible that more homogeneity in clinical presentations might be needed to detect whole brain volume differences in patients with bipolar disorder and healthy controls.

8.1.2.3.3 *Bipolar Disorder vs and Schizophrenia*

In this study I did not find any difference in whole brain volume in patients with bipolar disorder when compared to patients with schizophrenia. There is very limited data comparing whole brain volume between these disorders. Most studies report regional differences using whole brain volume to correct for volumetric differences accounted for by differences in brain size. However, previous studies have reported no differences in whole brain volume between patients with schizophrenia and patients with bipolar disorder (Harvey et al., 1994, Zipursky et al., 1997). Moreover, these results have been supported by a recent meta-analysis of 98 structural neuroimaging studies of patients with bipolar (Kempton et al., 2008). The authors reported a meta-analytical comparison of 121 patients with bipolar disorder and 236 patients with schizophrenia from 5 studies and found no differences in whole brain volume

between the two groups. Therefore, my finding of lack of whole brain volumes differences between schizophrenia and bipolar is in line with previous studies.

8.2 Familiarity: Brain volumes comparisons

8.2.1 *Patients with schizophrenia and bipolar disorder, their relatives and healthy volunteers*

For this analysis, the relatives of patients with psychosis were included. To my knowledge, there is no other study that has compared grey and white matter volume in these groups looking at familiality in a combined sample of scans obtained with different MRI protocols. Moreover, most previous studies focused their hypothesis on specific brain regions as a way to understand the pathophysiology of psychosis. In contrast, in this study the hypotheses were not related to the physiopathology of psychosis, but rather to their potential as biomarkers. This has been, to my knowledge, the first study that has looked at brain differences in such a sample, and also the first one that has assessed the potential of global volumes rather than regional volumes as possible biomarkers.

In this study, I found no significant differences between patients with schizophrenia and the group of relatives of these patients. Additionally, I found no differences between healthy volunteers and the relatives of patients with schizophrenia. McDonald and colleagues evaluated part of my sample, from the Maudsley Family Study. Here, similarly to my findings, when compared to healthy volunteers the relatives of patients with schizophrenia did not show any volumetric difference in brain structures (McDonald et al, 2006). However, the authors did not report global grey, white or whole brain matter volumes. In addition, the findings of my study are consistent with those of a more recent and large study using VBM methods that explored differences in brain volumes in schizophrenia, their non-affected siblings and healthy controls

(Boos et al., 2011). The authors found no differences in global volumes between the relatives and the patient or control groups.

Few studies have looked at global grey, white and whole brain matter volumes in the relatives of patients with schizophrenia (Cannon et al. 2002b; McDonald et al., 2002; Gogtay et al., 2003; Boss et al., 2007, 2011). The results of these studies have been contradictory. A recent study by Boos and colleages (2011) found no differences in global grey and white matter volumes between relatives of patients with schizophrenia and healthy volunteers. Moreover, a study by Goldman et al. (2009) found reduction of total grey matter thickness in patients with schizophrenia but in their relatives when compared to healthy controls. However, a meta-analysis by Boos and colleagues (2007) showed that whole gray matter volume in relatives of patients with schizophrenia was significantly smaller than the volume of healthy volunteers. The authors also described a trend for smaller white matter and whole brain volumes in the relatives group than in controls. However, they also described a significant heterogeneity in effect size across individual studies, which make the results less comparable. In addition, previous studies described structural brain changes found in schizophrenia such as accelerated grey matter loss seem to be also progressive in their unaffected relatives (Brans et al., 2008). These findings would support the evidence for grey matter reduction in relatives of schizophrenia found in Boos et al (2007) meta-analysis. However, it is worth mentioning that meta-analyses tend to have a larger statistical power to detect the more subtle differences that relatives of patients with schizophrenia seem to present. Therefore, sample size might be a reason for the lack of morphometric differences I was able to observe in my sample.

Nevertheless, my study is quite innovative in describing global brain volumes in a rather large sample. Additionally, although grey, white and whole brain volumes can be reliable biomarkers when comparing cases and controls, the

subtle differences conferred by genetic loading in their relatives might need a more specific marker.

In my study, patients with bipolar disorder showed significantly smaller whole brain volume than their relatives. Very few studies have described brain volume differences between patients with bipolar disorder and their relatives and as in schizophrenia, most studies have described regional differences (Marshall et al., 2004; McDonald et al., 2006). However, a twin study in bipolar disorder by van der Schot et al. (2009) described that in both the affected and non-affected co-twin group presents with smaller white matter volumes than healthy twins and reported these differences as related to genetic loading. On the other hand, the grey matter reductions found in both the affected and non-affected co-twins seem to be related to environmental factors. In addition, these authors specifically assessed the effect of lithium and described that this seems to attenuate both white and grey matter volume reductions in the affected co-twins. In my study, I did not find a significant difference when grey and white matter volumes were analyzed separately. However, when added together to produce a measure of whole brain matter volume, this measure was significantly smaller in the bipolar disorder patients in comparison to their relatives. This might be the result of subtle additive changes in different brain areas that only when added together become significant.

The comparison between relatives of patients with bipolar disorder and healthy volunteers did not highlight any signifficant difference in any of the three brain volumes I analized. These findings are consistent with those of previous studies (McIntosh et al., 2004; 2005; McDonald et al., 2006). In addition, a number of studies that explored brain volume differences in patients with bipolar disorder, their relatives and healthy controls were based on twin populations, mainly evaluating the genetic influence accounted for brain volume differences found in bipolar disorders (Kieseppa et al., 2003; van der Schot et al., 2009). However, these studies have also described brain

volume differences between the unaffected co-twin and healthy volunteers. Kieseppa et al (2003) reported a significant reduction in white matter volumes in the affected co-twins but no significant differences between the unaffected co-twins and the healthy volunteers. In the study by van der Schot et al., (2009) as described above, the unaffected co-twins had smaller grey and white matter volumes than the healthy twins, suggesting a genetic liability in these brain structures. However, the paper does not report if the difference was statistically signifficant, making it difficult to make any comparison with my findings.

8.2.2 *Familiality of brain volumes*

Structural brain volumes can be explored as quantitative traits. These traits show significant variation in healthy human populations as well as in patients with psychiatric disorders like psychosis. One way of understanding these differences might be by looking at the influence that genetic factors have on them. These, in combination with environmental factors, could help identifying the biological mechanisms underlying neuroanatomical phenotypes and the possible identification of risk factors for psychosis. In previous studies heritability estimates have shown a strong genetic component contributing to neuroanatomical phenotypes (Posthuma et al. 2002; Kremen at al., 2010). Brain volumes seem to have substantial heritability, with rates ranging from 70 to 95% for total brain volume, cerebellar, gray and white matter volumes, and corpus callosum (Kaymaz and Van Os, 2009). The higher the heritability rate, the less likely it is that the environment may have a prominent influence on the development of a particular phenotype. Therefore, looking at highly heritable structural brain phenotypes might provide an endophenotype for genes that are involved in the regulation of brain volumes in psychosis.

Brain volumes, particularly smaller whole brain and grey matter volumes, have been accepted as possibly reliable endophenotypes for psychotic disorders (Glahn et al., 2007; Prasad and Keshavan, 2008). However, these changes seem to be related not only to the presence of the illness, but also to the

presence in unaffected relative of a patient with psychosis (McDonald et al, 2002 and 2006; Gottesman et al., 2003; Prasad and Keshavan, 2008). Therefore, in this study I aimed to examine the role of familial risk in measures of brain volume. The sample I used has several advantages. First, it comprises a large pool of MRI and genetic data. Second, it includes the relatives of patients with two major psychotic disorders, namely schizophrenia and bipolar disorder. This has allowed me to look at the familiality of brain volumes. To explore whether brain volumes represent a familial trait, I compared the within-family variance against the between-family variance using a mixed model analysis. This method has been successfully used to estimate the familiality of clinical symptoms in schizophrenia (Wickham et al., 2001) and to assess possible endophenotypes in psychosis (Bramon et al., 2004, 2005). I found that for both, schizophrenia and bipolar disorder, family clusters accounted for 48% of the global grey matter total variance. For both disorders the findings reached statistical significance. In previous studies, grey matter volume reductions have been shown to be also present in the unaffected relatives of patients with schizophrenia (Cannon et al 1998a; McDonald et al, 2002 and 2006; Boos et al., 2007). These results support my finding and previous studies that proposed grey matter volume as a plausible endophenotype for psychosis (McDonald et al., 2004b; Kaymaz and Van Os, 2009).

Interestingly, belonging to the same family accounted for 43% of the total variance of white matter volume for subjects with bipolar disorder, but only for 27% of variance in families with schizophrenia. Although in both groups the results were statistically significant, this was more significant for the families in the bipolar group. These results are in correspondence with recent studies supporting a genetic influence in white matter reduction in both disorders (McIntosh et al., 2005; Hulshoff Pol et al., 2012). However, white matter changes, such as increased white matter hyperintensities (Altshuler et al., 1995; Videbech et al., 1997; Kempton et al., 2008; Beyer et al., 2009) have been more extensively reported in patients with bipolar disorder than in

patients with schizophrenia (Rivkin et al. 2000). Moreover, white matter hyperintensities have been also reported as present in relatives of bipolar disorder patients (Hajek et al., 2005). Therefore, my findings of greater familiality for white matter changes in patients with bipolar disorder than in schizophrenia might reflect differences in genetic liability for white matter in these disorders.

In conclusion, grey matter volume seems to be a useful biomarker for psychosis in general, while white matter volume appears more specific for bipolar disorder in which it shows substantial familial influence.

8.3 Brain volume and genes

There is evidence that normal brain development is greatly influenced by genetic factors (Lenroot and Giedd, 2008). In psychosis, brain development has been suggested to deviate from normal development (DeLisi et al., 1997; Liberman et al., 2001). It has been hypothesized that this might be due to a genetic predisposition in patients with psychosis (Brans et al., 2008). Changes occurring in neurodevelopment in subjects with psychosis might lead to the reduction of about 2% of brain volume consistently described in comparison to matched healthy controls (Wright et al, 2000). As discussed in previous sections, a number of studies in patients with schizophrenia have reported smaller volume of not only whole brain, but also of grey matter in comparison to healthy controls (Wright et al 2000; Steen et al., 2006, Tanskznene et al., 2009). This reduction seems to be also evident in adolescent onset schizophrenia, supporting the theory of schizophrenia as a neurodevelopmental disorder (Rapoport at al., 1999). In addition, subjects at high risk of developing the disorder because of their positive history for psychosis present similar changes, suggesting that genetic loading may play a role in the brain volume changes reported in psychosis (Lawrie at al., 1999; 2001). This is also consistent with evidence that relatives of patients with schizophrenia show grey matter volume reduction when compared to healthy

controls (McDonald et al, 2002 and 2006, Boos et al., 2007). Whole brain volume heritability has been estimated to be between 66% and 97% (Sullivan et al., 2003; Peper et al., 2007; Kaymaz and Van Os, 2009). In addition, twin studies have supported the theory of genetic influence in morphometric brain differences in psychosis by showing smaller whole brain volume in both co-twins with schizophrenia and in non-schizophrenic co-twins when compared to healthy control twins (Baare et al., 2001; van Haren et al., 2004). Furthermore, the progressive loss of grey matter described in patients with schizophrenia has also been shown in their healthy co-twins and seems to be greatly influenced by a genetic component (Brans et al., 2008).

Despite the strong evidence for genetic influence in the development of psychosis, there is a lack of consistent replication in candidate gene association studies so far (Harrison et al., 2003; Craddock et al., 2005b, 2009). This lack of replication has been interpreted as related to insufficient statistical power.

It has become widely accepted that the aetiology of psychosis is based on a combination of both complex genetic and environmental factors (Sullivan et al., 2003; Braff et al. 2007a). The most recent approach used to elucidate the pathways into these disorders has been based on the exploration of potential endophenotypes. Moreover, in recent GWAS studies a meta-analytical approach was used to increase the power to detect genome-wide significance associations (Shi et al., 2009; Scott et al., 2009; McMahon et al., 2010; Vassos et al., 2012). These results further highlight the importance of sample size when trying to disentangle a role for genetic influence in complex diseases such as psychosis.

Besides sample size, other sources for conflicting results in genetic association studies might lay on the studies' samples heterogeneity or due to the overlapping symptoms that schizophrenia and bipolar disorders present

with (Ivleva et al., 2007). This heterogeneity might be responsible for overlapping evidence of susceptibility genes across schizophrenia and bipolar disorder, such as DAOA (G72), DTNBP1, COMT, BDNF, DISC1, and NRG1 (Craddock et el., 2006). In addition, there is also evidence that gene expression might be influenced by the effect of environmental factors adding further heterogeneity to the sample characteristics and the endophenotypes tested (Caspi et al., 2002; Caspi & Moffit 2006).

In this project I looked at a number of candidate genes that have been described to be associated with brain volume changes in patients with psychosis. In addition, I looked at genes that have been described as implicated in brain development, to assess the potential influence in the brain changes described so far. My aim was to evaluate if specific SNPs could account for the brain volume differences I identified across groups.

I found that three SNPs collected from GWAS showed brain volume differences between patients and healthy controls. Patients homozygous for DTNBP1rs1047631-thymidine (TT) presented with smaller grey, white and whole grey matter volumes than healthy volunteers with the TT genotype. These results show that DTNBP1 has an effect in global brain volumes and this is supported by previous reports showing DTNBP1 mRNA to be widespread in the brain. In addition, DTNBP1 mRNA is reduced in patients with schizophrenia compared to healthy volunteer's areas such as the dorsolateral prefrontal cortex (Weickert et al., 2004). There is also evidence that smaller hippocampal volume in patients with schizophrenia relative to healthy volunteers might be due to the reduced DTNBP1 protein expression in the brain during its formation (Weickert et al., 2008). These findings suggest a potential role of DTNBP1 in neurodevelopment in schizophrenia and support my findings of smaller brain volumes in patients with psychosis than in healthy volunteers.

Another gene that showed positive association with brain volumes was OLIG2. OLIG2 is expressed throughout the brain during neurodevelopment and in adulthood with the main function of regulating the differentiation between motor neurones and oligodendrocytes (Ono et al 2009). Moreover, it is also involved in the glial reconstitution after brain injury (Ono et al 2009). In patients with schizophrenia, OLIG2 expression has been shown to be reduced (Gerogieva et al 2006; Mitkus et al., 2008). The OLIG2-rs10590004 allele A has particularly been associated to schizophrenia (Gerogieva et al 2006) and patients with psychotic symptoms in obsessive compulsive disorder (Stewart et al., 2007). In my study, the results showed that patients homozygous for OLIG2 (CC) showed smaller whole white matter and whole brain volume than healthy controls with the CC genotype. A recent study by Prata et al (2012) showed altered white matter integrity in healthy volunteers with OLIG2 allele A carriers. However, the authors did not report white matter volume assessment in their report. The difference in risk allele A reported by Gerogieva et al (2006) and Prata et al. (2012), and my finding of C allele as the risk allele, might be related to C allele being more specific to brain volumes and psychosis. Thus, the C allele might be the risk allele for reduced white and whole brain volume in patients with psychosis but not in healthy volunteers. This is supported by a study by Huang et al (2008) who found a possitive association with C allele in patients with schizophrenia with the OLIG2 proxy (rs762178) I used in my study. To my knowledge this is the first study that examines the genetic variation of OLIG2 on brain volumes in patients with psychosis. Therefore, the evidence that OLIG2 is associated with schizophrenia, brain volume reductions in healthy volunteers and in patients with psychosis suggests its potential role in brain development in both groups and requires further investigation.

In addition, I found that patients homozygous for MCPH s1057090-cytosine (CC) showed significantly smaller white matter and, at trend level, smaller whole brain volume than healthy volunteers. None of the other MCPH SNPs

evaluations showed a significant association with brain volumes in any of the groups assessed. MCPH has been shown to be associated with the brain volume reduction found in primary microcephaly (Jamieson et al., 2000; Pattison et al., 2000; Moynihan et al 2000). Due to the role of MCPH in the development of smaller brains, it has been hypothesized that MCPH might influence brain volume in the general population and possibly in psychosis. One previous study reported larger cranial volume in males in Chinese population associated with MCPH rs1057090 (Wang et al 2008). However, others have failed to find associations between MCPH and brain volume in healthy populations (Woods et al., 2006; Dobson et al., 2007). To my knowledge there are only a few studies evaluating MCPHs effects in patients with psychosis and they have reported conflicting findings. Rivero et al. (2006) reported negative results while Rimol et al (2009) found a significant association on brain volume reductions in male patients with psychosis.

Despite the described positive associations of the above three SNPs with brain volumes, none of the SNPs reached statistical significance after Bonferroni test for multiple testing. As described above, the most likely reason for this lack of association seems to be related to the sample size.

Sample size does not seem to be the only issue interfering when assessing genetic associations. This was shown by the failure to replicate previous findings for several candidate genes in a large European Sample, which included about 4000 cases (Sanders et al., 2008). This result contradicts previous predicted differences from earlier and smaller samples. The reasons for these contradictory results have been discussed by Ioannidis (2008). The author highlights that there are a number of factors leading towards false positive findings, such as inflated effect sizes due to thresholds selection and suboptimal power as shown in simulated power calculation for a study or; selective reports. More recently, it has become more generally accepted that genetic studies on average require large sample sizes, estimated between

1,000 and 3,000, to have the power to detect small to medium genetic effects (OR of 1.1-1.3) (Tiwari et al., 2010; Gejman et al., 2010; Byerdley and Bradner 2011). On the other hand, the use of large sample sizes might in itself lead to phenotypic heterogeneity and population stratification resulting in spurious associations (McCarthy et al., 2008).

There is a number of ways to increase the statistical power to detect real associations in genetic studies of complex genetic diseases. For example, by increasing the sample size, the power to detect associations directly increases. Another way is to use measures that increase the effect size. This is done by either choosing an accurate and specific phenotype and/or genotyping a specific region of interest (Evans and Purcell, 2012). In my study I have applied these principles to explore associations between selected genes and the specific phenotypes. However, for the 13 SNPs genotyping data from the original 387 samples of European origin, less than half of the sample passed QC, thus by significantly reducing the number of participants available for analysis and therefore, its statistical power.

The decision to include data from GWAS in my study was based on the evidence that GWAS has the advantage of low-cost, high-density genotyping and large, well-characterized sample sets (Turner et al., 2011). With this approach, I would have been able to obtain genotyping data from 13 SNPs in around 380 subjects at a lower financial cost than if the genotyping was done for each SNP separately. However, in the GWAS approach, because of the inclusion of very large samples, false-negative results may be increased by failure to control for experimental factors such as low-quality DNA samples, poorly performing SNP assays, and errors in sample identification, leading to artefacts in the system and reduced power (Turner et al., 2011). These samples therefore require a stricter QC. Undergoing the strict QC is very important for genome wide association studies, where large sample sizes are needed to detect small effects and hundreds of thousands of polymorphisms

are studied. Due to the inclusion of very large samples, GWAS might be more able to afford a reduction in sample size without losing statistical power. However, the candidate gene approach I used in my study was shown to have a great impact on the reduction of sample size by using GWAS genotyping data. I believe that in my study this was particularly true as samples had been collected and stored over many years, and also used for genotyping other genes and therefore, affecting the quality and quantity of DNA (Pompanon et al., 2005). All these factors might have affected the quality and quantity of DNA samples used for the GWAS genotyping, leading to the reduction of almost three quarters of my sample. In addition, the assessment of population structure in the GWAS study identified about 20 out of the 387 participants that had been wrongly identified as Caucasian. Therefore, those subjects were also excluded from the genotyping possibly compromising the statistical power of the sample.

In this study I also explore the potential role of RELN gene in brain morphometry in psychosis. RELN has been associated with schizophrenia (Shifman et al., 2008, Liu et al. 2010) and bipolar disorder (Fatemi et al., 2000; Guidotti et al., 2000; Goes et al., 2010). In my study I found not association between RELN and total grey and white matter volume as well as whole brain volume as probable endophenotypes. To my knowledge this is the first time that RELN has been evaluated against these putative endophenotypes. These results concur with a previous study which had evaluated an association of RELN with intermediate phenotype such as measures of brain structure, brain function, and gene expression and found a not significant association (Tost et al., 2010). However, other studies have reported association between working memory and the RELN gene in patients with schizophrenia (Wedenoja et al., 2009). This finding continues to support the hypothesis that RELN is implicated in the pathway of the development of psychosis however; it might not be implicated in brain structural changes but in neuropsychological endophenotypes such as working memory.

In addition, in my study I also included some of the most frequently reported candidate genes for psychosis such as BDNF, NRG and COMT, which were genotyped with a smaller scale genotyping method (i.e. at the SGDP or by commercial companies). There are previous reports of association of regional brain volume differences between psychosis and healthy volunteers with BDNF (Szeszko et al., 2005; Ho et al., 2006); COMT (Ohnishi T. et al, 2006) and NRG1 (Mata et al., 2009). However, in my study I found that none of these genes were associated with total brain volume. The lack of positive associations between these genes and brain volume might be related to the choice of different endophentypes from previous studies as they have mostly used regional rather than global brain volume measures. However, a study by Dutt et al. (2009), which included some participants from my sample, also failed to find an association between previously reported reductions in hypothalamus volume and BDNF (Szeszko et al., 2005). The lack of consistency in gene-morphometry associations might be related to the use of different endophenotypes, methodology used to measure brain volume, sample heterogeneity or again, to an underpowered sample for the genetic analysis.

The issue of statistical power creates another challenge when looking at genetic association with endophenotypes such as brain structure. Most neuroimaging studies may be powered at sample sizes as little as 10–20 subjects to detect between-group differences, but these require strong *a priori* hypotheses to show statistically significant differences between patients and healthy controls (Friston et al., 1999). In the case of whole brain volume and global grey and white matter volume, these requirements are not always fulfilled. In this study, for the genetic analysis, I had run a power calculation. The results were that with genes with 1% allele frequency, α of 0.003 and a sample of above 350 participants, I would have had 80% power to detect 4% of the variance in brain volumes. This power would have allowed me to detect

brain volume differences as described in the literature (reductions in psychosis at around 2% (Wright et al., 2000). In addition to my calculations, a number of candidate gene studies have reported significant associations between a certain SNP and brain volume changes, with samples sizes similar to mine. These studies included samples between 100 and 400 subjects of healthy volunteers (Zinkstok at al., 2006; Pezawas et al., 2004), and sometimes even smaller samples of patients with psychosis (n=78) (Addington et al., 2007), (n=51) (Zinktok et al., 2008) and in patients (n=19) compared to controls (n=25) (Szeszko et al. 2005). However, these studies have not been consistently replicated seeding doubts about their truthness.

Analyses of statistical power can sometimes shed light on study results, particularly in the interpretation of negative results or lack of replication such as mine. Statistical power is considered the most pertinent factor that influences findings in genetic studies particulalrly in the context of modern whole-genome association studies, in which issues of coverage and multiple testing are dominant. Power analysis can either be done prospectively or retrospectively. Typically, a prospective power analysis is conducted prior to the research study, to estimate the sample size required to achieve adequate power. In my study I carried out a prospective power analysis. Differently, a retrospective power analysis is conducted after a study has been completed using the obtained sample and effect sizes to determine what the power was in the study, assuming the effect size in the sample was equal to the effect size in the population. Most GWAS studies take the approach of a retrospective power analysis, while introducing the risk of what is called the "power approach paradox". Here, a study with a null result is considered to show more evidence that the null hypothesis is actually true when the p-value is smaller, since the apparent power to detect an actual effect would be higher. In fact, a smaller p-value is properly understood to make the null hypothesis less likely to be true. Therefore, special attention should also be paid to GWAS positive results.

Besides altering the critical value, there are two other ways to increase the power of a statistical test: increasing the size of the sample on which the GWAS approach is being used, and by increasing the effect size. In my study I was not able to increase the sample size since the neuroimaging data had been obtained in the past and I could not enlarge the sample. Therefore, I sought to increase the effect size by selecting a specific phenotype. However, as described before, due to the number of SNPs tested, the power to detect statistically significant effects was potentially affected by to the adjustments for multiple corrections

Some of the SNPs of SBNO1, HMGA2 genes assessed in this study have been only described to influence brain volume in healthy individuals (Taal et al., 2012, Stain et al., 2012). SBNO1 showed an effect on brain growth in early life (Taal et al., 2012) while HMGA2 gene, that encodes for protein that regulates stem cell renewal during neurodevelopment (Nishino et al., 2008), was associated with development of intracranial volume in healthy adults (Stein et al., 2012). In my study I did not find an association between these genes and brain volume therefore it is possible that they are not specifically implicated in the brain changes described in psychosis. In addition, a recent GWAS study also described an association between CRHR1 and brain volume in a healthy population (Ikram et al., 2012). Corticotrophin-release hormone (CRH) is released during stress and it is thought to create changes in the synaptic spines stability leading to possible brain cortical malfunctioning and the development of depression and psychoses (Bennett, 2008). Moreover, CRF has been found to be increased in the cerebrospinal fluid of patients with post-traumatic stress disorder with psychotic symptoms (Sautter et al., 2003). These findings suggest a potentail role of CRHR1 gene for brain volume changes in psychosis. However, in my study, I have not found an association between CRHR1 and any of the brain volume assessed in any of the groups.

To my knowledge no previous studies have looked at the potential role of SBNO1, HMGA2 and CRHR1 in psychosis and as mentioned above is possible that they are not specifically implicated in the brain changes described in psychosis. In addition, these genes have been identified as part of GWAS studies where a larger number of participants (about 9000) was included. These studies therefore had more statistical power to detect associations than my current sample.

Finally, another factor to consider when exploring possible reasons for the lack of significant findings in the genetic analysis might be related to the characteristics of the sample I used, which is in many ways atypical. For instance, I combined subjects at their first presentation with patients in chronic stages of the illness. The current literature suggests that whole brain volumes as well as global grey and white matter volume changes are present at all stages, and even before the illness outbreak. However, meta-analyses suggest that these changes are considerably subtler in the early illness stages, as shown by Steen and colleagues (2006), and therefore the overall effect might have been diluted in my sample. On the other hand, there is also evidence that medications might play a role in brain morphology in patients, even after relatively short periods of treatment (Dazzan et al 2005, Lieberman et al, 2005; Ho et al., 2011). Therefore, medications might act as confounding factors obscuring results. Moreover, I combined patients with schizophrenia and bipolar disorder as I was not able to evaluate them separately due to small sample size. Although there is evidence of a genetic and brain morphometric overlap between these two illnesses, it is possible that again some of the differences between the two diagnoses have affected the results, in terms of carrying different genetic vulnerability. Therefore, the heterogeneity of the sample might have blurred the association between specific SNPs and their impact on brain volumes.

In conclusion, although I was able to detect genetic association between three SNPs and patients with psychosis, this loss significance after multiple testing

corrections was applied. In addition, although I used data from the GWAS, which allows the evaluation of millions of SNPs, my approach was that of a candidate gene for brain volume in psychosis, and the analysis of such association was limited to subjects with structural MRI collected with two particular protocols. Although large, my sample could still have been underpowered to detect such associations.

8.4 Strengths of the study

Combination of neuroimaging data increases the statistical power to detect brain volume differences and its genetic influences. In this study I was the only rater of quality control and processing of the images. Additionally, I was blind to subject status. Also, I pre-processed all the images with the same pipeline. The pre-processing and analysis of a large neuroimaging sample was possible thanks to the application of a semi-automated method, SPM8. The combination of all these factors increased the consistency and made some of the most discussed pitfalls of multicentre studies less likely to occur. Another advantage in comparison to other studies that have combined multiple samples, is that the all the subjects were recruited from the same catchment area. This, to a certain extend, makes the effect of potential confounders such as environmental factors that could influence genetic expression less likely. Finally, one of the advantages of the design I used is that I included the relatives of patients affected with different psychotic illnesses. This approach increases the power to detect familiality of certain traits, and to assess the potential influence of genetic loading of brain volume deviations from the general population.

8.5 Limitations

There are a number of limitations in the current study. The neuroimaging data obtained for this study were acquired over a period of about 6 years. It has been described that with time, MRI scanners quality declines due to changes in magnetic fields strength, and this changes the quality of the images

obtained. Therefore, this might have had an impact on the pre-processing and the segmentation processes that were used to obtain grey and white matter volumes. Additionally, given that the brain undergoes changes with age, the large variability in age in the sample makes the interpretation of findings more difficult. Also, as for age, the influence of genetic and environmental factors on brain volumes may change with time, making the interpretation of genetic influence less reliable in the current study design.

The assessment of familiarity in this study takes into account the variability of certain traits within families, in comparison to the trait variability in not related individuals. Although this model cannot differentiate genetic from environmental factors, it is thought to estimate the genetic influence in the assessed traits (Bramon et al., 2004; 2005). This differs from a more specific approach taken by family and twins studies. In both of these models a phenotypic variance is studied to assess the contribution of genetic and environmental factors. Therefore, although I showed that the familial contribution to the brain volume differences was statistically significant, I was not able to draw any conclusion about the extent of genetic *versus* environmental contributions. Additionally, in my study I did not have enough statistical power to explore the allelic familial risk due to small sample size. This should be investigated in larger samples, which would allow distinguishing genetic variations in patients and their relatives. This would enable us to understand how potential risk alleles for psychosis are clustered in families. Moreover, although I found a positive association in three genotypes and brain volume and psyhsis, the sample was too small and the data underwent multiple testings. Therefore, these suggesting results should be further explore with a larger sample assessing specific SNPs to ensure more accurate results.

Another limitation of the study has been related to the fact that my project involved merging and using data from 3 different research groups, with a setup agreement already in place on data sharing. Because of this, I had difficulties in gathering additional information on all the variables that could have

potentially acted as confounders in my analysis. This is important because the apparent relationship or lack of it, between an independent variable and a dependent variable may in fact be related to a confounding factor. The presence of this apparent result is called omitted-variable bias, and potentially creates a biased effect estimates.

Should I have had access to more data, I would have liked to include detailed information on different type of medication exposure (i.e. number and type of anti-psychotics, antidepressants and lithium; cumulative dose of medication since onset); duration and severity of illness and use of recreational drugs. The addition of these potentially relevant variables would have been appropriate as it could have increased the sensitivity of the analysis. However, increasing the number of comparisons conducted to account for confounders can in itself affect the statistical power to detect statistical significant results, as the significance threshold would need to be corrected in order to reduce the risk of type I error (i.e. difference found by chance).

Lastly, although VBM has become widely used, this method comes with some pitfalls. For example, it is sensitive to the pre-processing stage. The reliability of VBM in combining different protocols was tested in the calibration study. I found that modulated images provide more robust and reliable correlation of volumes obtained with different protocols. In addition, although SPM is a fully automated method that allows to easily analyzing large number of neuroimaging data reducing researcher bias, it also has some disadvantages. For instances, the pre-procesing semi-automatic program runs intensity non-uniformity correction during the segmentation pre-processing. Therefore, if applying N3 algorithm to ensure better segmentation (Acosta-Cabronera et al. 2008), some of the default SPM should be modified to avoid double correction. This is particularly important when analyzing images that have been obtained over a long period of time (Sled at al., 1998). Finally, small differences in acquisition parameters can play an important role when

analyzing multicentre or multi-study scans. However, I believe that despite these limitations, this study supports the use of combined neuroimaging data from different datasets to achieve a better understanding of brain changes in psychosis and as a strategy to identify endophenotypes.

8.6 Final conclusion and future research

This study provide evidence that neuroimaging data acquired with different protocols can be reliably combined depending on the parameters used; in fact, special attention should be paid to the MRI acquisition parameters as I showed that these can greatly influence the possibility of combining data. Adequate sampling and considering a symptom dimension rather than a categorical approach may help reduce the heterogeneity of clinical presentation. Similarly, achieving an appropriate sample size is important to localize relevant genes involved in pathological variation in brain volumes according to different symptoms dimensions. In addition, future neuroimaging analyses of this sample should include the assessment of regional volumes, of areas such as the pre-frontal cortex and the medio-temporal lobes. These regions have been shown to be highly heritable and to be involved in the neuropathophysiology of psychosis. In addition, in future studies it will be also important to further assess the effects of medication in the brain volume particular white matter which has been less extensively explored in structural neuroimaging studies.

More specifically, as mentioned above, one of the limitations of the genetic analysis in this study has been the power to detect statistical signifficant results. I was unable to increase the number of subjects included in the study as the MRI had already been already collected. In order to increase the statistical significance of the tests, in future work I will explore specific brain areas to provide more accurate phenotyping, for example through genotyping a specific region of interest or adding certain co-variates to refine the results. For instance, since I found an association (albeit not significant) between

whole white matter volume and OLIG2, in my future work, I would like to further explore this results with a region of interest approach specifically evaluating the prefrontal cortex in patients with bipolar disorders and patients with schizophrenia separately.

In addition, it is now well establish that complex disorders such as psychosis arise from a combination of genetic and environmental factors and that the interaction between the different factors may be important. If two variables of interest interact, the relationship between each of the interacting variables and the dependent variable depends on the value of the other interacting variable. Therefore, it is more difficult to predict the consequences of changing the value of a variable. However, if this interaction is included in the model one can control for this obtaining more reliable results.

Some of the environmental factors of relevance for the development of psychosis are the use of recreational drugs. It has been reported that the use of cannabis may increase risk of schizophrenia, as suggested by a number of systematic reviews (Arseneault et al., 2004 and Moore et al., 2007). Moreover, brain morphology has been considered to be useful as an intermediate phenotype in genetic research in psychiatric disorders (Baare et al., 2001; Durston et al., 2005). Therefore, another area of interest for my future research is the evaluation of gene-environment interaction. I will explore interactions between genes such as COMT and the use of recreational drugs like cannabis, exploring the effect of this interaction in the Pre-Frontal Cortex in my study sample.

Finally, in my future work I will use a polygenic score analysis approach. This method has recently created interest for assessing the explanatory power of an ensemble of markers. This approach is therefore most suited to addressing some of the power limitations implicit to my study that were discussed above. From the data obtained from the GWAS analysis on an initial training sample, I

will be able to rank markers on the neurodevelopmental genes I included in my thesis according to their evidence for an association, usually with their P-values. Then, an independent replication sample is analysed by constructing, for each subject, a polygenic score consisting of the weighted sum of its trait-associated alleles, for a subset of the top ranking markers. Once these ranks are obtained, two related but distinct applications of this score are then possible. Firstly, testing for an association between the score and the trait in the replication sample can determine whether associated markers reside within those contributing to the score. Secondly and perhaps more usefully, the polygenic score can be used to predict individual trait values such as whole brain volumes. Effect sizes are estimated for each marker and can be used to construct a polygenic score for each subject in an independent replication sample. The score is tested for association in the replication sample, in which the tested trait may differ from that in the training sample. One of the advantages of using the polygenic score is that it reduces the number of tests needed in the analysis. As a result, this method allows limiting or reducing threshold of strictness to reject a null hypothesis in order to compensate for the multiple comparisons, which was one of the limitations of my study that could potentially obscur the results.

An alternative approach could rely on the fact that very recently, at the International Conference of Psychiatric Genetics in October 2012, it was announced that a large collaboration involving approximately 25,000 people with schizophrenia and 28,000 controls had identified 62 risk genes for schizophrenia. Although not yet published, this should allow the construction of a polygenic score which can be used to summarise the degree of genetic loading, and this score then related to brain volumes. Finally, although the limitation of measurable endophenotypes for genetic associations continues, this study provides evidence to support the use of multiple protocols or multi-centre scanners that would enable to increase the sample size for this type of genetic analysis.

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Appendix 1

Psychosis Screening Questionnaire

PSYCHOSIS SCREENING QUESTIONNAIRE (PSQ)

_____

Interviewer …….. Date……

AESOP ID ☐ ☐ ☐ ☐ ☐

Interviewer……..

_____

Code: No = 0 Unsure = 1 Yes = 2

In this health survey we have to ask about a whole range of experiences. Some of these experiences are quite rare. However, I would be very much obliged if you would bear with us and answer the questions I am going to ask you now.

Q1. Over the past year, have there been times when you felt very happy indeed without a break for days on end? ☐

(a) Was there an obvious reason for this? ☐

(b) Did your relatives or friends think it was strange or complain about it? ☐ **If 2 stop**

Q2. Over the past year, have you ever felt that your thoughts were ☐

directly interfered with or controlled by some outside force or person?

(a) Did this come about in a way that many people would find hard to believe, for instance through telepathy? ☐ **If 2 stop**

No = 0 Unsure = 1 Yes = 2

Q3. Over the past year, have there been times when you felt that people were against you? ☐

(a) Have there been times when you felt that people were deliberately acting to harm you or your interests? ☐

(b) Have there been times when you felt that a group of people was plotting to cause you serious harm or injury? ☐ **If 2 stop**

Q4. Over the past year have there been times when you felt that something strange was going on? ☐

(a) Did you feel it was so strange that people would find it very hard to believe? ☐ **If 2 stop**

Q5. Over the past year, have there been times when you heard or saw things that other people couldn't ☐ **If 1 or 2 stop**

(a) Did you at any time hear voices saying quite a few words or sentences when there was no-one around that might account for it? ☐ **If 2 stop**

Q6. Have you ever received treatment for any psychiatric or psychological problem?

……

Appendix 2
Handedness

Handedness
(Expanded version)

Respondent ID _____ Name of Interviewer _____

Date of Interview _____

ANNETT HAND PREFERENCE QUESTIONNAIRE

HAND:
Ask subject to demonstrate (tick hand used to perform):

1. Writing	**R**	**L**	**7. Sweeping**	**R** **L**
2. Throwing a ball	**R**	**L**	**8. Shovelling**	**R** **L**
3. Hammering nail	**R**	**L**	**9. Unscrewing a jar lid**	**R** **L**
4. Brushing teeth	**R**	**L**	**10. Dealing cards**	**R** **L**
5. Striking match	**R**	**L**	**11. Threading a needle**	**R** **L**
6. Holding racquet	**R**	**L**	**12. Holding scissors**	**R** **L**

FOOT:
Ask subject to demonstrate:

1. Kicking football **R** **L**

EYE:

1. Hold pen vertically at arms length and line up tip with point in distance with both eyes open, then cover each eye in turn and see if still lined up.
Tick side that, when covered, prevents pen from being aligned **R** **L**

2. Fold sheet of paper as if it was a telescope, and ask subject to use it to look at a distance.
Tick eye used. **R** **L**

The definition of the Classes of Hand Preference

Actions	*Preference Classes*
A. Writing*	**1. Right (or R+E) for all actions**
B. Throwing*	**2. Left for any of F, G, H only**
C. Using a racquet*	**3. Left for L and no others except above**
D. Striking a match*	**4. Left for I and no others except above**
E. Cutting with scissors	**5. Right writing but left for any other primary action**
F. Threading a needle	**6. Left writing but right for any other primary action**
G. Sweeping with broom	**7. Left for all primary actions but right for any others**
H. Shovelling with long-handled shovel	**8. Left (or L+E) for all actions**
I. Dealing playing cards	
K. Using a toothbrush*	
L. Unscrewing a jar	

(Revised from Annett 1976) *Primary actions: those most highly intercorrelated (Annett, 1970)

Appendix 3

Family Interview for Genetic Studies: FIGS

FIGS Family Tree

This page left intentionally blank for Family Tree to include first degree relatives with date of birth, gender, and place of birth

FIGS General Screening Questions

Interview date: | | | | — | | | — | | | | |
Month *Day* *Year*

Family last name: _____ ___ *Family ID Number:* | | | | |

Informant name: _____ ___ _____ *ID:* | | | | | |
_ _ __
First *MI* *Last*

INTERVIEWER: Before you begin, you need to generate or obtain a family tree, with date of birth and gender, on which to record all of the responses to the following General Screening Questions. (See FIGS Manual for details.)

Step 1: *Let's go over your family.* (Include parents, siblings and offspring aged 18 or above)

Step 2: *Now I am asking you to keep in mind all those in your family as I go through this list of questions.* (Note all positive responses on the pedigree.)

Did anyone:

Feel very low for a couple of weeks or more, or have a diagnosis of depression?

Attempt or complete suicide?

Seem overexcited (or manic) day and night, or have a diagnosis of mania?

Have visions, hear voices, or have beliefs that seem strange or unreal?

Have unusual or bizarre behavior, or have a diagnosis of schizophrenia?

(Was anyone) hospitalized for psychiatric problems?

Step 3: For each of these given a positive response in the General Screening, complete the symptom checklist for any suspected: Depression/Mania, Psychosis, or Paranoid/Schizoid/Schizotypal Personality.

FIGS Depression checklist

Interview date: ☐☐☐ — ☐☐ — ☐☐☐☐
Month *Day* *Year*

Informant name: _____ ___ _____ *ID:* ☐☐☐☐☐
First *MI* *Last*

Person being described name: _____ ___ _____ *ID:* ☐☐☐☐☐

Relationship to informant *Do B*

Code for a single episode (best recalled, worst episode if possible).

	No	Yes	Unk
1. *During depression…*			
1.a) *…was he/she depressed most of the day, nearly every day, for as long as a week or more?*	0	1	9
1.b) *…did he/she lose interest in things or become unable to enjoy most things, for as long as a week?*	0	1	9
1.c) *…did he/she have a change in appetite or weight without trying to?*	0	1	9
1.d) *…did he/she have a change in sleep patterns (either too much or too little)?*	0	1	9
1.e) …did he/she become unable to work, go to school, or take care of household responsibilities?	0	1	9

If yes: Describe:

_____

_____

_____

1.f) *…did he/she move or speak more slowly than usual?*	0	1	9
1.g) *…did he/she pace or wring his/her hands?*	0	1	9
1.h) *…did he/she have less energy or feel tired out?*	0	1	9
1.i) *…did he/she feel guilty, worthless or blame himself/herself?*	0	1	9
1.j) *…did he/she have trouble concentrating or making decisions?*	0	1	9
1.k) *…did he/she talk of death or suicide? Or try suicide?*	0	1	9

1.l) *…did he/she have visions, or hear voices, or have beliefs or behavior that seem strange or unusual, at the same time as (symptoms above)?* (If **YES**, complete a Psychosis Checklist after this one.) 0 1 9

Code Response

2. Code and describe professional treatment:

0 1 2 3 4 9

0. None

1. Inpatient:
_____

2. Outpatient:
_____

3. ECT:
_____

4. Medication:
_____

9. Unknown

3. Age of onset — Age

4. Number of episodes — Episodes

5. Duration of longest episode in weeks — Weeks

Code Response

6. Rate and code impairment or incapacitation:

0 1 2 3 4 9

0. None
1. Modified RDC Impairment
2. Modified RDC Incapacitation
3. RDC Minor Role Dysfunction
4. Change from previous functioning
9. Unknown

7. Interviewer judgement on reliability of this information:

1 2 3

1. Good
2. Fair
3. Poor

FIGS Mania checklist

Interview date: ___ — ___ — ____ *Month* *Day* *Year*

Informant name: _____ ___ _____ *ID:* | | | | | |

First *MI* *Last*

Person being described name: _____ ___ _____ *ID:* | | | | | |

Relationship to informant *Do B*

	No	Yes	Unk
1. *For most of the time day and night over several days, did he/she (more than usual)…*			
1.a) *…seem too happy/high/excited?*	0	1	9
1.b) *…become so excited or agitated it was impossible to converse with him/her?*	0	1	9
1.c) *…act very irritable or angry?*	0	1	9
1.d) *…need less sleep without feeling tired?*	0	1	9
1.e) …show poor judgement (e.g., spending sprees, sexual indiscretions?)	0	1	9

If yes: Describe:

_____

_____

____

1.f) *…behave in such a way as to cause difficulty for those around him/her* (obnoxious/manipulative)*?*	0	1	9
1.g) *…feel that he/she had special gifts or powers?*	0	1	9
1.h) *…become more talkative than usual?*	0	1	9
1.i) *…jump from one idea to another?*	0	1	9
1.j) *…become easily distracted?*	0	1	9
1.k) *…get involved in too many activities at work or school?*	0	1	9
1.l) *…have visions? Hear voices? have beliefs or behavior that seem strange or unusual? at the same time as (above symptoms)?* (If **YES**, complete a Psychosis Checklist after this one.)	0	1	9

Code Response

2. Code and describe professional treatment: 0 1 2 3 4 9

   0. None

   1. Inpatient:
   _____

   2. Outpatient:
   _____

   3. ECT:
   _____

   4. Medication:
   _____

   9. Unknown

Age

3. Age of onset | | |

Episodes

4. Number of episodes | | | |

Weeks

5. Duration of longest episode in weeks | | | |

Code Response

6. Rate and code impairment or incapacitation: 0 1 2 9

   0. None
   1. Impaired
   2. Incapacitated
   9. Unknown

7. Interviewer judgement on reliability of this information: 1 2 3

   1. Good
   2. Fair
   3. Poor

FIGS Psychosis checklist

Interview date: | | | | — | | | — | | | | |

Month *Day* *Year*

Informant name: _____ ___ _____ *ID:* | | | | | |

First *MI* *Last*

Person being described name: _____ ___ _____ *ID:* | | | | | |

Relationship to informant *Do B*

Code for a single episode (best recalled, worst episode if possible).

1. *What were his/her unusual beliefs or experiences?*
Specify:

_____
_____77
_____

Did he/she ever…		No	Yes	Unk
1.a)	*…believe people were following him/her, or that someone was trying to hurt or poison him/her?*	0	1	9
1.b)	*…believe someone was reading his/her mind?*	0	1	9
1.c)	*…believe he/she was under the control of some outside person or power or force?*	0	1	9
1.d)	*…believe his/her thoughts were broadcast, or that an outside force took away his/her thoughts or put thoughts into his/her head?*	0	1	9
1.e)	*…have any other strange or unusual beliefs?*	0	1	9

If yes: Describe:

_____
_____
_____

1.f)	*…see things that were not really there?*	0	1	9
1.g)	*…hear voices or other sounds that were not real?*	0	1	9

If yes: Describe:

_____
_____
___ [ ]

		No	Yes	Unk
1.g.1)	(Code **YES** if: voice with content having no relation to depression or elation, or voice keeping up running commentary on subject's behavior or thoughts, or two or more voices conversing.)	0	1	9
1.h)	*...speak in a way that was difficult to make sense of?*	0	1	9

If yes: Describe:

_____

_____

____

1.i)	*...seem to be physically stuck in one position, or move around excitedly without any purpose?*	0	1	9
1.j)	*...appear to have no emotions, or inappropriate emotions?*	0	1	9

2. *How long did the longest of these experiences last?* Weeks [ ][ ][ ]

INTERVIEWER: If less than 1 week (unless successfully treated), STOP HERE. Otherwise continue, if informant is knowledgeable about this person.

INTERVIEWER: If subject did NOT have any episode of Major Depression or Mania (by FIGS checklists from this informant), skip to question 6.

	No	Yes	Unk
3. When any (SX above) happened, did he/she also have the mood disturbance we discussed before, at the same time?	0	1	9

INTERVIEWER: For the rest of this checklist, "illness duration" refers to total time of illness, including active and prodromal and/or residual symptoms and/or treatment (include time on medication).

	No	Yes	Unk
4. (Probe and code **YES** if mania and/or depression lasted at least 30% of total duration of illness described above, or medication for it.)	0	1	9
5. (Probe and code **YES** if illness described above, or medication for it, was ever present for as long as one week, without depression and/or mania.)	0	1	9

5.a) (Code **YES** if the above was true for as long as two weeks.) 0 1 9
Code Response

6. Code and describe professional treatment (Code and describe all that apply): 0 1 2 3 4 9

0. None

1. Inpatient:
_____

2. Outpatient:
_____

3. ECT:
_____

4. Medication:
_____

9. Unknown

Describe details and/or other treatment:

7. Age of onset — Age

8. Number of episodes (Code **001** if chronic symptoms and/or treatment since onset) — Episodes

9. Total illness duration (all episodes, includes active and prodromal and/or residual symptoms and/or treatment. — Weeks / Years

Code Response

10. Rate and code impairment or incapacitation: 0 1 2 9
0. None
1. Impaired
2. Incapacitated
9. Unknown

11. Interviewer judgement on reliability of this information: 1 2 3
1. Good
2. Fair
3. Poor

INTERVIEWER: If informant apparently does not know subject well enough to give information on Prodromal/Residual symptoms, STOP HERE.
If duration criterion for DSM III-R Schizophrenia, Chronic Type, already met, (question 9, total illness duration > 2 years), STOP HERE.

FIGS Psychosis checklist

INTERVIEWER: Use this page only if Schizo-affective is ruled out (by questions 3 to5 above), or if the psychosis symptoms lasted at least one week (or shorter duration if successfully treated).

Establishing the Prodromal Period:

16. *Now I would like to ask you about the year before his/her* (**psychotic symptoms)** *started. During that time did he/she…*

(Ask after completing question 16.a-n for the Prodromal period:)

Establishing the Residual Period:

Now I would like to ask you about the year after his/her (**psychotic symptoms)** *stopped. During that time did he/she…*

	Prodromal Period			**Residual Period**		
	No	Yes	Unk	No	Yes	Unk
16.a) …*stay away from family and friends, become socially isolated?*	0	1	9	0	1	9
16.b) …*have trouble doing his/her job, going to school, or doing work at home?*	0	1	9	0	1	9
16.c) …*do something peculiar like talking to self in public?*	0	1	9	0	1	9
16.d) …*neglect hygiene and grooming?*	0	1	9	0	1	9
16.e) …*appear to have no emotions or inappropriate emotions?*	0	1	9	0	1	9
16.f) …*speak in a way that was hard to understand, or was he/she at a loss for words?*	0	1	9	0	1	9
16.g) …*have unusual beliefs or ideas?*	0	1	9	0	1	9
16.h) …*have unusual perceptions, like sensing the presence of a person not actually present?*	0	1	9	0	1	9
16.i) …*have no interests, no energy?*	0	1	9	0	1	9
16.j) …*find special meaning in TV, radio, or newspaper articles?*	0	1	9	0	1	9
16.k) …*feel nervous with other people?*	0	1	9	0	1	9
16.l) …*worry that people were out to get him/her?*	0	1	9	0	1	9
		Weeks				

17.a) *How long did he/she have these experiences?* | | | |

INTERVIEWER: Return to top of question 16 to establish the Residual period and code in Residual Column.

Weeks

17.b) *How long did he/she have these experiences after his/her* **(Active psychotic features)** *stopped?* | | | |

	No	Yes	Unk
18. *Was he/she always this way?*	0	1	9

Code based on Informant's Report:

Did person being described have:			No	Yes	Unk
1.	**Depression**		0	1	9
1.a)	Single		0	1	9
1.b)	Recurrent		0	1	9
1.c)	Impaired/Incapacitated		0	1	9
1.d)	Treatment		0	1	9
				Age	
1.e)	Age of onset				
2.	**Mania**		0	1	9
2.a)	Single		0	1	9
2.b)	Recurrent		0	1	9
2.c)	Impaired/Incapacitated		0	1	9
2.d)	Treatment		0	1	9
				Age	
2.e)	Age of onset				
3.	**Psychosis**		0	1	9
3.a)	(1) Chronic or (2) acute?		1	2	
3.b)	Outside of mood disorder		0	1	9
3.c)	Treatment		0	1	9
				Age	
3.d)	Age of onset				

Complementary tables

Complementary table 1: Genotyping frequencies

Genotyping groups according to frequencies for statistical analysis

Genotype	patient	relative	control
NRG 221533			
CC/CT	63	70	41
TT	37	43	18
NRG 241930			
GG	37	40	18
GT/TT	58	64	31
NRG 243177			
CC	32	33	19
CT	56	53	29
TT	21	10	15
DTNBP1P1757			
AA/AG	31	44	14
GG	45	58	29
DTNBP1P1320 rs760761			
CC	35	53	24
CT/TT	28	33	15
BDNF rs6265			
AA/AG	39	32	26
GG	94	82	54

COMT rs4680			
AA	37	24	23
AG	62	58	39
GG	32	30	17
DTNBP1_rs1047631			
CC/CT	13	15	7
TT	48	48	20
NTRK2_rs10868219			
CC/CT	32	35	11
TT	27	28	15
RELN_rs7341475			
AA/AG	16	17	9
GG	45	46	18
OLIG2_rs1059004_rs762178			
CC	25	14	8
CT/TT	35	44	19
MCPH1_rs2305022			
AA	35	40	13
AC/CC	26	23	14
MCPH1_rs930557_rs2440416			
CC	37	41	14

CG/GG	24	22	12
MCPH1_rs1057090_rs2912057			
AA/AG	36	39	17
GG	23	23	10
MCPH1_rs2912016_rs2959798			
CC/CT	35	37	14
TT	26	26	12
ASPM_rs3762271_rs1360558			
CC/CT	49	51	18
TT	12	12	9
SBNO1_rs7980687_rs12322888			
AA/AG	23	26	8
GG	38	37	19
HMGA2_rs1042725			
CC	19	17	12
CT/TT	41	46	14
CRHR1_rs11655470			
AA/AG	40	44	14
GG	20	19	13
NRG 221533			
CC/CT	63	70	41
TT	37	43	18

NRG 241930			
GG	37	40	18
GT/TT	58	64	31
NRG 243177			
CC	32	33	19
CT	56	53	29
TT	21	10	15
DTNBP1P1757			
AA/AG	31	44	14
GG	45	58	29
DTNBP1P1320 rs760761			
CC	35	53	24
CT/TT	28	33	15
BDNF rs6265			
AA/AG	39	32	26
GG	94	82	54
COMT rs4680			
AA	37	24	23
AG	62	58	39
GG	32	30	17

Complimentay table 2: Genotype frequencies in patients and healthy volunteers and Hardy–Weinberg equilibrium assessment results

Gene/alleles	Patient n=152	Healthy volunteers n=117	Hardy–Weinberg equilibrium Chi square- χ^2 (df,N), p value
NRG 221533	100	59	χ^2(df2,N=159)=4.23,p=.12
CC	9	12	
CT	54	29	
TT	37	18	
NRG 241930	95	49	χ^2(df2,N=144)=.444,p=.80
GG	37	18	
GT	48	24	
TT	10	7	
NRG 243177 rs6994992	109	63	χ^2(df2,N=172)=.633,p=.73
CC	32	19	
CT	56	29	
TT	21	15	
DTNBP1P1757rs2005976	76	43	χ^2(df2,N=119)=3.69,p=.16
AA	6	0	
AG	25	14	
GG	45	29	
DTNBP1P1320 rs760761	63	39	χ^2(df2,N=102)=3.94,p=.14
CC	35	24	

CT	22	15	
TT	6	0	
BDNF rs6265	133	80	χ^2(df2,N=213)=.363,p=.83
AA	4	2	
AG	35	24	
GG	94	54	
COMT rs4680	131	79	χ^2(df2,N=210)=.234,p=.89
AA	37	23	
AG	62	39	
GG	32	17	
DTNBP1_rs1047631			χ^2 (df2,N=87)=4.57,p=.10
CC	0	2	
CT	13	5	
TT	48	20	
DTNBP1_rs6937379			χ^2 (df2,N=86)=.7.53,p=.02
CC	29	20	
CT	23	6	
TT	9	0	
NTRK2_rs10868219			χ^2 (df2,N=84)=1.24,p=.54
CC	7	2	
CT	25	9	
TT	27	15	
RELN_rs7341475			χ^2 (df2,N=87)=1.03,p=.60
AA	1	0	

AG	15	9	
GG	45	18	
OLIG2rs762178			χ^2 (df2,N=86)=1.37,p=.50
CC	25	8	
CT	20	12	
TT	15	7	
MCPH1_rs2305022			χ^2 (df2,N=87)=3.85,p=.15
AA	35	13	
AC	24	10	
CC	2	4	
MCPH1_rs2440416			χ^2 (df2,N=86)=4.07,p=.13
CC	37	14	
CG	22	8	
GG	2	4	
MCPH1_ rs2912057			χ^2 (df2,N=85)=.47,p=.78
AA	9	3	
AG	27	14	
GG	23	10	
MCPH1_ rs2959798			χ^2 (df2,N=86)=.08,p=.96
CC	9	4	
CT	26	10	
TT	26	12	
ASPM_ rs1360558			χ^2 (df2,N=87)=1.92,p=.38
CC	19	8	

CT	30	10	
TT	12	9	
SBNO1_ rs12322888			χ^2 (df2,N=87)=1.17,p.555
AA	3	2	
AG	20	6	
GG	38	19	
HMGA2_rs1042725			χ^2 (df2,N=86)=1.27,p=.53
CC	19	12	
CT	26	9	
TT	15	5	
CRHR1_rs11655470			χ^2 (df2,N=87)=2.43,p=30
AA	7	4	
AG	33	10	
GG	20	13	